MGIEasy

RNA Exome Instructions

Instruction Version: 1.0

Kit Version:

MGIEasy RNA Directional Library Prep Set（1000006385） V2.1

MGIEasy Exome Capture V5 Probe Set（940-000187-00） V1.0

MGIEasy Exome Capture Accessory Kit（1000007743）V1.0

Revision History

Manual Version	Date	Description
1.0	Dec. 2021	◆ Update Cat. No.

Note: Please download the latest version of the manual and use it with the corresponding kit.

Search manual by Cat. No. or product name from website:

https://en.mgi-tech.com/download/files.html

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Chapter 1 Application Description

1.1 Introduction

The MGIEasy RNA Exome application is specifically designed for constructing RNA coding region libraries for the MGI high-throughput sequencing platform series. The reagents for this RNA capture method include MGIEasy RNA Directional Library Prep Set, MGIEasy Exome Capture V5 Probe Set and a MGIEasy Exome Capture Accessory Kit.

This user guide provides an RNA coding region capture method that has been optimized for poor quality and degraded RNA samples. 10 - 100 ng of degraded human total RNA can be converted into customized libraries for sequencing on an MGI sequencing platform listed below.

1.2 Application

This protocol is suitable for RNA derived from Human primary samples. It describes the process in which Total RNA is converted into stranded libraries and achieves the capture of the transcriptome coding regions using the MGIEasy Exome Capture V5 probe set.

1.3 Platform Compatibility

Constructed libraries are compatible with

BGISEQ-500 (PE100)
MGISEQ-2000 (PE100), DNBSEQ-G400 (PE100)

1.4 Reagents

The reagents for this RNA capture method include MGIEasy RNA Directional Library Prep Set, MGIEasy Exome Capture V5 Probe Set and a MGIEasy Exome Capture Accessory Kit. Further information on Cat. No., Components and Specifications are listed below.

Table 1 MGIEasy RNA Directional Library Prep Set (16 RXN) (Cat. No.: 1000006385)

Modules & Cat. No.	Components	Color-Coded Screw Cap	Spec& Quantity
MGIEasy RNA Directional Library Prep Kit Cat. No.: 1000005270	Fragmentation Buffer	Green	93 μL/tube× 1 tube
	Directional RT Buffer 1	Green	88 μL/tube× 1 tube
	Directional RT Buffer 2	Brown	5 μL/tube× 1 tube
	RT Enzyme Mix	Green	24 μL/tube× 1 tube
	Directional Second Strand Buffer	Yellow	470 μL/tube× 1 tube
	Second Strand Enzyme Mix	Yellow	78 μL/tube× 1 tube
	ERAT Buffer	Orange	132 μL/tube x 1 tube
	ERAT Enzyme Mix	Orange	55 μL/tube x 1 tube
	Ligation Buffer	Red	450 μL/tube x 1 tube
	DNA Ligase	Red	34 μL/tube x 1 tube
	PCR Enzyme Mix	Blue	470 μL/tube x 1 tube
	PCR Primer Mix	Blue	90 μL/tube x 1 tube
	UDG	Blue	21 μL/tube× 1 tube
MGIEasy DNA Adapters-16 (Tube) Kit Cat. No.: 1000005284	DNA Adapters	White	10 μL/tube× 16 tubes
MGIEasy DNA Clean Beads Cat. No.: 1000005278	DNA Clean Beads	White	8 mL/tube× 1 tube
	TE Buffer	White	4 mL/tube× 1 tube
MGIEasy Circularization Module Cat. No.: 1000005260	Splint Buffer	Purple	186 μL/tube x 1 tube
	DNA Rapid Ligase	Purple	8 μL/tube x 1 tube
	Digestion Buffer	White	23 μL/tube x 1 tube
	Digestion Enzyme	White	42 μL/tube x 1 tube
	Digestion Stop Buffer	White	120 μL/tube x 1 tube

Table 2 MGIEasy Exome Capture V5 Probe Set (16 RXN) (Cat No.: 940-000187-00)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Exome Capture V5 Probe Kit Cat. No.: 1000007741	MGI Exome V5 Probe	Black	80 μL/tube × 1 tube
MGIEasy Exome Capture Hybridization and Wash Kit (Box 1) Cat. No.: 940-000168-00	Block 1	Yellow	40 μL/tube × 1 tube
	Block 2	Yellow	40 μL/tube × 1 tube
	Block 5	Yellow	8 μL/tube × 1 tube
	Hyb Buffer 3	Green	64 μL/tube × 1 tube
MGIEasy Exome Capture Hybridization and Wash Kit (Box 1) Cat. No.: 940-000169-00	Hyb Buffer 1	Green	160 μL/tube × 1 tube
	Hyb Buffer 2	Green	7 μL/tube × 1 tube
	Hyb Buffer 4	Green	90 μL/tube × 1 tube
	Binding Buffer	White	12800 μL/bottle × 1 bottle
	Wash Buffer I	White	8000 μL/bottle × 1 bottle
	Wash Buffer II	White	24000 μL/bottle × 1 bottle

Table 3 MGIEasy Exome Capture Accessory Kit (16 RXN) (Cat. No.: 1000007743)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Exome Capture Accessory Kit Cat. No.: 1000007743	Post-PCR Enzyme Mix	Blue	800 μL/ tube × 1 tube
	PCR Primer Mix	Blue	96 μL/ tube × 1 tube
	Block 3	Yellow	16 μL/ tube × 1 tube
	Block 4	Yellow	160 μL/ tube × 1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy RNA Directional Library Prep Kit

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice

MGIEasy DNA Adapters Kit

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice

MGIEasy Circularization Kit

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice

MGIEasy Exome Capture Accessory Kit

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice

MGIEasy Exome Capture Hybridization and Wash Kit (Box 1)

- Storage Temperature: -25°C to -15°C
- Transport Conditions: transported on dry ice.

MGIEasy Exome Capture Hybridization and Wash Kit (Box 2)

- Storage Temperature: room temperature
- Transport Conditions: transported at room temperature

MGIEasy Exome Capture V5 Probe Kit

- Storage Temperature: -80°C
- Transport Conditions: transported on dry ice

MGIEasy DNA Clean Beads

- Storage Temperature: 2°C to 8°C
- Transport Conditions: transported with ice packs

*Production Date and Expiration Date: refer to the label

* Please ensure that an abundance of dry ice remains after transportation.

* Under appropriate transport, storage, and usage conditions, performance of products is guaranteed up until the displayed expiration date.

1.6 Equipment and Materials Required but not Provided

Table 4 Equipment and Materials Required but not Provided

Equipment	Vortex Mixer Desktop Centrifuge Thermocycler Magnetic rack DynaMag™-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent Qubit™ 3 Fluorometer (Thermo Fisher Scientific™, Cat. No. Q33216) Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA) Eppendorf Concentrator（Eppendorf, Cat. No. 5305000398） Thermomixer or water bath equipment Nutator or other nutating mixer/shaker Magnetic rack for 96-well plate (BioMag, Cat. No. BMB-96）or equivalent
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) RNase Zap（Ambion, Cat. No. AM9780） DNase I（NEB, Cat. No. M0303S）（optional） 100% Ethanol (Analytical Grade) Qubit™ RNA HS Assay Kit（Invitrogen, Cat. No. Q32852）（optional） Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212) Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854) High Sensitivity DNA Kits (Agilent Technologies™, Cat. No. 5067-4626) Agilent DNA 1000 Kit (Agilent, Cat. No. 5067-1504) Agilent RNA 6000 Pico Kit（Agilent, Cat. No. 5067-1513）（optional） Dynabeads™ M-280 Streptavidin（Invitrogen, Cat. No. 112.06D）
Consumables	1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C) 0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 8-Strip PCR tubes (Axygen, Cat. No. PCR-0208-C) or 96-well plate (Axygen, Cat. No. PCR-96M2-HS-C) 2.0 mL centrifuge tubes (Axygen, Cat. No. MCT-200-C) or equivalent 8 Strip Domed Caps Fit 0.2 mL PCR Tube Strips（Axygen, Cat. No. PCR-02CP-C）or equivalent Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856) or equivalent Filter Tips（Axygen, Cat. No. TF-100）or equivalent Clear Adhesive Film (ABI, Cat. No. 4306311) Blade or knife

1.7 Precautions and Warning

- Instructions provided in this manual are intended for general use only and may require optimization for specific applications. We recommend adjusting according to the experimental design, sample types, sequencing application, and other equipment.
- Before RNA related processing, ensure the entire work area and equipment is cleaned using RNase Zap and 80% Ethanol. This includes cleaning all pipettes, tube racks, and small equipment. Wear mask, appropriate gloves and lab coats.
- Prior to starting, remove the reagents from storage, and prepare them for use: For enzymes, keep in storage until required, centrifuge briefly and place on ice until further use. For other reagents, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice until further use.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermocyclers with heated lids for reactions. Before placing the samples, preheat the thermal cycler and heated lid to the initial reaction temperature.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR Products and may decrease the accuracy of results. Therefore, we recommend physically separating two working areas in the laboratory for PCR reaction preparation and PCR product cleanup, respectively. Use designated equipment for each area and clean regularly to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment)
- If you have other questions, please contact MGI technical support MGI-service@mgi-tech.com

Chapter 2 Sample Preparation

2.1 Sample Compatibility and Requirement

This protocol is applicable for total RNA derived from human samples, particularly for total RNA from FFPE and fresh frozen tissues. It is recommended to use 10 - 100 ng total RNA as input.

2.2 Sample Quality Control

Before proceeding with library preparation, use an Agilent 2100 Bioanalyzer to perform quality control checks of RNA samples. DV_{200} can be used for assessing the quality of RNA samples and determining the condition of library construction (Table 5). The DV_{200} indicates the proportion of RNA fragments larger than 200 nt in the sample. Take the analysis result from Agilent 2100 Bioanalyzer trace below as an example for the DV_{200} calculation. Detailed calculation is shown in Figure 1:

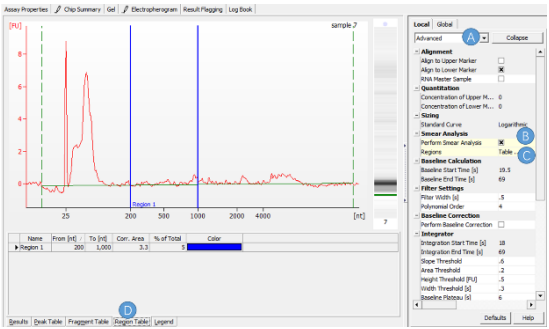

Figure 1 The Calculation of DV_{200}

A: In the Agilent 2100 Bioanalyzer result figure, choose *Advanced* under *Local*

B: Check the *Perform Smear Analysis* option under *Smear Analysis*

C: Double-click on *Table* to enter the range of fragments to be calculated. The figure shows the example of *from* 200 bp *to* 1000 bp.

D: Obtain the proportion of selected fragment shown as *% of Total* in the *Region Table*

Table 5 Recommended Conditions for Library Construction from Different Quality Samples

DV_{200} Value	Recommended amount of total RNA input	Conditions of fragmentation	PCR cycles
＞70%	10 ng	94℃，8 min	15
50-70%	20-50 ng	94℃，8 min	15
30-50%	50-100 ng	94℃，6 min	15
＜30%	100 ng (with a risk of failure of NGS library construction)	No fragmentation	16

- RNA purity: $OD_{260/280}$=1.8 to 2.0.
- If DNA contamination is found in RNA sample, perform DNase I digestion to remove DNA before proceeding to the next step in the procedure, please refer to Appendix A.
- While not recommended, processing <10 ng RNA as input into library preparation can be attempted, however this may lead to a decreased yield of PCR product and lower comparison rate in analysis result.

Chapter 3 Library Construction Protocol

This protocol has been optimized using 50 ng total RNA (UHRR, Universal Human Reference RNA) with DV_{200} > 70%. If a different quality of RNA input is used, please identify optimal input and fragment conditions by adjusting the recommended conditions in Table 5.

3.1 RNA Fragmentation

⚠ **Note: In the following procedures, do not vortex. Please use pipette to mix solution.**

3.1.1 Add 50 ng total RNA sample to a 0.2 mL PCR tube and add NF Water to a total volume of 10 μL.

3.1.2 Add 4 μL of Fragmentation Buffer to the 10 μL RNA sample in step 3.1.1 and gently pipette mix 10 times. Briefly centrifuge and place the tube into the thermocycler. Follow Table 6 below for fragmentation conditions.

Table 6 The Recommended Condition of RNA Fragmentation

Insert Size	Temperature of RNA Fragmentation	Time of RNA Fragmentation
150 bp	94°C	8min

3.1.3 When the reaction completes, immediately place the tube on ice for 2 minutes. Centrifuge for 10 seconds and immediately proceed to Reverse Transcription.

3.2 Reverse Transcription and Second Strand Synthesis

⚠ **Note: In the following procedures, do not vortex. Please use pipette to mix solution.**

3.2.1 Remove Directional RT Buffer 2 from -20°C storage and thaw it at room temperature. Vortex to mix and centrifuge briefly to collect the solution to the bottom of the tube. Make a 1:16 dilution of Directional RT Buffer 2, as shown in Table 7 below.

Table 7 Dilution (1:16) of Directional RT Buffer 2

Components	Volume
Directional RT Buffer 2	1 μL
NF water	16 μL
Total	17 μL

⚠ **Note: The diluted Directional RT Buffer 2 should be used immediately. Please discard any unused diluent. Prepare fresh diluted Directional RT Buffer 2 for each library construction.**

3.2.2 Remove Directional RT Buffer 1 from -20°C and thaw it at room temperature. Invert several times to mix. Prepare the Reverse Transcription mixture on ice (see Table 8):

Table 8 Reverse Transcription Mixture

Components	Volume
Directional RT Buffer 1	4 μL
Diluted Directional RT Buffer 2	1 μL
RT Enzyme Mix	1 μL
Total	6 μL

3.2.3 Transfer 6μL of Reverse Transcription Mixture to the RNA Fragmentation product in step 3.1.3. Pipette up and down 10 times to mix. Centrifuge briefly to collect the solution at the bottom of the tube.

3.2.4 Place the PCR tube in step 3.2.3 into the thermocycler and run the following program in Table 9:

Table 9 The Reaction Conditions of Reverse Transcription

Temperature	Time
Heated lid	On
25°C	10 min
42°C	30 min
70°C	15 min
4°C	Hold

3.2.5 Once the reaction completes, place the product on ice and centrifuge for 10 seconds.

3.2.6 Remove Directional Second Strand Buffer from -20°C and thaw at room temperature. Invert several times to mix. Prepare the Second Strand Synthesis mixture on ice (see Table 10):

Table 10 Second Strand Synthesis Mixture

Components	Volume
Directional Second Strand Buffer	26 μL
Second Strand Enzyme Mix	4 μL
Total	30 μL

3.2.7 Transfer 30 μL Second Strand Synthesis mixture to the Reverse Transcription product from step 3.2.5. Pipette 10 times to mix and centrifuge briefly to collect the solution at the bottom of the tube.

3.2.8 Place the PCR tube from step 3.2.7 into the thermocycler and run the following program in Table 11:

Table 11 Reaction Conditions of Second Strand Synthesis

Temperature	Time
Heated lid	On
16°C	60 min
4°C	Hold

3.2.9 Once the reaction completes, place the product on ice and centrifuge for 10 seconds. Transfer all Second Strand Synthesis product to a new 1.5 mL tube and place it on ice for the next reaction.

✔ **Stopping Point: Second strand synthesis product can be stored at -20°C up to 16 hours.**

3.3 Cleanup of Second Strand Synthesis Product

⚠ **Note: Please read Appendix B carefully before you begin.**

3.3.1 Remove DNA Clean Beads from the refrigerator and bring to room temperature for 30 min beforehand. Vortex and mix thoroughly before use.

3.3.2 Use pipette to transfer 75 μL of DNA Clean Beads to the Second Strand Synthesis product in step 3.2.9. Gently pipette up and down at least 10 times to mix thoroughly. Ensure that all the solution and beads are expelled from the tip into the tube before discarding.

3.3.3 Incubate at room temperature for 5 minutes.

3.3.4 Centrifuge briefly and place the 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until liquid becomes clear. Carefully remove and discard the supernatant using a pipette.

3.3.5 With the 1.5 mL tube on the Magnetic Separation Rack, add 200 μL freshly prepared 80% Ethanol to the tube without disturbing the beads. Incubate 30 seconds. Carefully remove and discard the supernatant.

3.3.6 Repeat step 3.3.5 once more. Remove all liquid from the tube without disrupting the beads. Centrifuge briefly to collect any remaining liquid at the bottom, separate beads magnetically and then remove remaining liquid using a small volume pipette tip.

3.3.7 Keep the 1.5 mL tube on the Magnetic Separation Rack with the lid open and allow the beads to air dry until no wetness (reflectiveness) is visible but before the pellet over dries and cracks.

3.3.8 Remove the 1.5 mL tube from the Magnetic Separation Rack and add 42 μL of TE Buffer to elute

the DNA. Gently pipette mix at least 10 times to mix thoroughly and centrifuge briefly.

3.3.9 Incubate at room temperature for 5 minutes.

3.3.10 Centrifuge briefly and place the 1.5 mL tube back onto the Magnetic Separation Rack for 2-5 minutes until liquid becomes clear. Transfer 40 µL supernatant to a new 0.2 mL PCR tube.

✔ **Stopping Point: Purified Second Strand Synthesis product can be stored at -20°C overnight.**

3.4 End Repair and A-tailing

3.4.1 Prepare the End Repair and A-tailing mixture in a new 0.2 mL PCR tube on ice (see Table 12).

Table 12 End Repair and A-tailing Mixture

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

3.4.2 Transfer 10 µL of the End Repair and A-tailing mixture to the 0.2 mL PCR tube from step 3.3.10. Vortex 3 times (3 s each) and briefly centrifuge to collect the solution at the bottom of the tube.

3.4.3 Place the 0.2 mL PCR tube from step 3.4.2 into the thermocycler and run the following program in Table 13.

Table 13 End Repair and A-tailing Reaction Conditions

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

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

⚠ **Warning: DO NOT STOP AT THIS STEP. Please continue to step 3.5.**

3.5 Adapter Ligation

Note: The amount of Adapter used in Adapter Ligation depends on the amount of RNA input. Please refer to Appendix C carefully before you proceed.

3.5.1 Following Table 14 below, make a 1:9 dilution of the Adapter. Mix and centrifuge briefly for further use.

Table 14 Adapter Dilution

Components	Volume
Adapter	1 μL
TE Buffer	9 μL
Total	10 μL

3.5.2 Please refer to the instructions for MGIEasy DNA Adapters (see Appendix C). Add 5 μL of diluted DNA Adapters to the PCR tube in step 3.4.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution to the bottom of the tube.

3.5.3 Prepare the Adapter ligation mixture in a new 0.2 mL PCR tube on ice (see Table 15).

Table 15 Adapter Ligation Mixture

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

3.5.4 Pipette slowly to transfer 25 μL of Adapter Ligation mixture to the PCR tube from step 3.5.2. Vortex 6 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

Note: Due to the viscosity of the Adapter Ligation mixture, please pipette slowly and ensure that the correct amount has been used.

3.5.5 Place the 0.2 mL PCR tube from step 3.5.4 into the thermocycler and run the following program in Table 16.

Table 16 Adapter Ligation Reaction Conditions

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

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

3.5.7 Add 20 µL TE Buffer, for a total volume of 100 µL and transfer all of the solution to a new 1.5 mL tube.

✔ **Stopping Point: Adapter-ligated DNA can be stored at -20°C for up to 16 hours.**

3.6 Cleanup of Adapter-Ligated DNA

⚠ **Note: Please read Appendix B carefully before you begin.**

3.6.1 Please refer to the instructions in Appendix B. Take out DNA Clean Beads from refrigerator and bring to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

3.6.2 Transfer 50 µL DNA Clean Beads to the sample tube from step 3.5.7. Pipette up and down at least 10 times to mix thoroughly. Ensure that the solution and beads are fully dispensed from the pipette tip into the tube before proceeding.

3.6.3 Incubate at room temperature for 5 minutes.

3.6.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.6.5 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.

3.6.6 Repeat step 3.6.5 once more, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the tube, separate the beads magnetically and then remove any remaining liquid using a small volume pipette.

3.6.7 Keep the 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 over-dry and crack.

3.6.8 Remove the sample tube from the Magnetic Separation Rack and add 22 µL TE Buffer to elute

the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.6.9 Incubate at room temperature for 5 minutes.

3.6.10 Centrifuge briefly and place the sample tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 20 µL supernatant to a new 0.2 mL PCR tube.

✔ **Stopping Point: After cleanup, Adapter-ligated DNA can be stored at -20°C up to 1 month.**

3.7 PCR Amplification

3.7.1 Prepare the PCR Amplification mixture on ice (see Table 17).

Table 17 PCR Amplification Mixture

Components	Volume
PCR Enzyme Mix	25 µL
PCR Primer Mix	4 µL
UDG	1 µL
Total	30 µL

3.7.2 Transfer 30 µL of PCR amplification mixture to the PCR tube from step 3.6.10. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.7.3 Place the PCR tube from step 3.7.2 into the thermocycler and run the following program in Table 18.

Table 18 Reaction Conditions for PCR Amplification

Temperature	Time	Cycles
Heated lid	on	
37°C	20 min	
95°C	3 min	
95°C	30 s	
56°C	30 s	15 cycles
72°C	1 min	
72°C	5 min	
4°C	Hold	

3.7.4 Once PCR completion, centrifuge briefly to collect the solution at the bottom of the tube. Transfer all the solution to a new 1.5 mL tube.

3.8 Cleanup of PCR Product

⚠ **Note: Please read Appendix B carefully before you begin.**

3.8.1 Take DNA Clean Beads from refrigerator and bring to room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

3.8.2 Transfer 50 μL DNA Clean Beads to the tube from step 3.7.4. Pipette up and down at least 10 times to mix thoroughly. Ensure that all liquid and beads are expelled from the pipette tip into the tube before proceeding.

3.8.3 Incubate at room temperature for 5 minutes.

3.8.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.8.5 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.

3.8.6 Repeat step 3.8.5 once more, remove all 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.

3.8.7 Keep the sample 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 over-dry and crack.

3.8.8 Remove the sample tube from the Magnetic Separation Rack and add **32 μL** TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.8.9 Incubate at room temperature for 5 minutes.

3.8.10 Centrifuge briefly then place the sample tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **30 μL** supernatant to a new 1.5 mL tube.

✔ **Stopping Point: After cleanup, purified PCR Products can be stored at -20°C.**

3.9 Quality Control of PCR Product

3.9.1 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 final PCR products is ≥ 250 ng.

3.9.2 Assess the fragment size distribution of purified PCR products with electrophoresis-based equipment such as a Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer™ (Advanced Analytical). Figure 2 shows the final size distribution is 150 bp-300 bp.

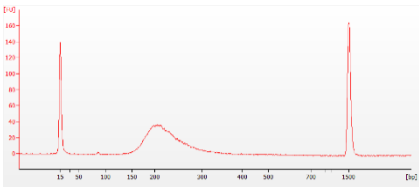

Figure 2 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR Product

3.10 Pre-Hybridization Preparation

⚠ **Note: A 96-well PCR plate is recommended for the hybridization reaction. Other steps can use PCR tubes or 8-Strip PCR tubes depending on the number of samples.**

3.10.1 If only one sample per hybridization reaction, transfer 250 ng of PCR products according to the concentrations measured in the last step. If multiplexing is performed to hybridization, 250 ng of each sample is required, and the total amount of PCR products should be between 500ng and 1000 ng. A 4-plex is the maximum pooling possible with this kit. **If starting with highly degraded FFPE samples, only one sample per hybridization reaction is recommended to ensure the hybridization efficiency is maximized**. For adapter and pooling strategies, please refer to Appendix C.

3.10.2 Prepare the Block mixture on ice (see Table 19).

Table 19 Block Mixture

Components	Volume
Block 1	2.5 μL
Block 2	2.5 μL
Block 3	1 μL
Block 4	10 μL
Total	16 μL

Note: Block 3 and Block 4 are reagents from the MGIEasy Exome Capture Accessory Kit, which are designed exclusively for the MGISEQ /DNBSEQ platform.

3.10.3 Transfer 16 μL of Block mixture to each of the PCR products from step 3.10.1 to prepare the Pre-Hybridization Mixture. Pipette up and down at least 10 times to mix thoroughly. Uncap and place the tubes containing the mixture on the concentrator, set the temperature to 65°C, vacuum and spin time for around 30min, until the final concentrated volume is 9 μL. If the final volume is less than 9 μL, add NF water into the concentrated product to reach a final volume of 9 μL.

3.10.4 Place the 9 μL of Pre-Hybridization Mixture from step 3.10.3 into the thermocycler and run the program in Table 20.

Table 20 Pre-hybridization Reaction Conditions

Temperature	Time
Heated lid	on
95°C	5 min
65°C	Hold

3.11 Hybridization and Capture

3.11.1 Prepare the Hybridization Mixture in a new 0.2 mL PCR tube (see Table 21).

Table 21 Hybridization Mixture

Components	Volume
Hyb Buffer 1	10 μL
Hyb Buffer 2	0.4 μL
Hyb Buffer 3	4 μL
Hyb Buffer 4	5.6 μL
Total	20 μL

3.11.2 Incubate the Hybridization Mixture from step 3.11.1 in the thermocycler at 65°C for at least 5 min.

Before use, ensure there is no precipitation in the mixture.

3.11.3 Prepare the Probe mixture on ice (see Table 22) in a new 96-well PCR plate (recommended).

Table 22 Probe Mixture

Components	Volume
NF water	1.5 μL
Block 5	0.5 μL
MGI Exome V5 Probe	5 μL
Total	7 μL

⚠ **Note: The MGI Exome V5 Probe must be thawed on ice and added last to the mixture.**

3.11.4 Tightly close the Strip Domed Caps on the 96-well PCR plate from step 3.11.3, then place the Probe Mixture into the thermocycler and run the program in Table 23.

Table 23 Probe Mixture Incubation

Temperature	Time
Heated lid	on
65°C	2 min
65°C	Hold

3.11.5 Keeping all the mixtures above (pre-hybridization mixture, hybridization mixture, and probe mixture) at 65°C, immediately transfer 13 μL of the Hybridization Mixture from step 3.11.2 into the 9 μL of Pre-Hybridization Mixture from step 3.10.4 at 65 °C. Pipette up and down to mix thoroughly.

⚠ **Note: 100 μL Filter Tips are recommended for this step.**

3.11.6 Transfer 22 μL of the combined pre-hybridization and hybridization mixtures from step 3.11.5 into the 96-well plate with 7 μL of the Probe Mixture from step 3.11.4. Pipette up and down to mix thoroughly.

⚠ **Note: 100 μL Filter Tips are recommended for this step.**

3.11.7 Cover the PCR plate with Clear Adhesive Film, press the film firmly against the plate to make sure all wells are completely sealed. Repeat this step to make sure film is completely sealed..

3.11.8 Keep the 96-well plate at 65 °C (set the heated lid of thermocycler to 105 °C) for hybridization for **24 hours** according to the program in Table 24.

Table 24 Hybridization Reaction Conditions

Temperature	Time
Heated lid (105°C)	On
65°C	Hold

3.12 Pre-Elution Preparation

3.12.1 Turn on the Thermomixer and set at 65 °C at least 30 min before beginning the elution process. For each hybridization reaction, add 1.8 mL of Wash Buffer II to a 2.0 mL tube and place it in the Thermomixer for preheating.

3.12.2 Vigorously vortex Dynabeads™ until evenly mixed. For each hybridization reaction, transfer 50 µL of Dynabeads™ to a new 2.0 mL tube.

3.12.3 Add 200 µL of Binding Buffer to each tube containing Dynabeads™ and vigorously vortex for 5 seconds to re-suspend the magnetic beads.

3.12.4 Centrifuge briefly and place the tube(s) onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.12.5 Repeat step 3.12.3 to 3.12.4 twice more.

3.12.6 Finally, add 200 µL of Binding Buffer to each tube containing Dynabeads™ to re-suspend the beads.

3.13 Elution

3.13.1 Keep the mixtures from step 3.11.8 on the thermocycler following the 24 hours incubation. Slice the adhesive film in-between each row of wells and carefully peel off the film row by row. Quickly use a pipette to estimate the remaining hybridization solutions one by one and transfer each mixture into a separate tube from step 3.12.6 containing prepared Dynabeads™.

⚠ **Note: If the volume of remaining hybridization solution is less than 19 µL, the yield may be low.**

⚠ **Note: While handling large number of samples, in order to reduce the vaporization of the hybridization solution, it is recommended to handle the samples in sets of six to eight reactions. In-between sets, the user should re-adhere the film and close the heated lid of the PCR thermal cycler cover for ten seconds before continuing with the next set of reactions.**

3.13.2 Fix the tubes from step 3.13.1 on a Nutator (or other similar mixer) for mixing by 360° rotations and incubate at room temperature for 30 min.

3.13.3 Take the tubes off the mixer.

3.13.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.13.5 Add 500 μL of Wash Buffer I, close the tube lid, and turn tubes upside down several times to re-suspend the bead mixture, then incubate at room temperature for 15 min.

3.13.6 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.13.7 Add 500 μL of pre-heated Wash Buffer II from step 3.12.1. Put the tubes in the Thermomixer and set the mixing speed to 1000 rpm. Press 'short' for 10 s to make sure all the beads are re-suspended, then set speed to 0 rpm. Incubate at 65 °C on the Thermomixer for 10 min.

3.13.8 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.13.9 Repeat step 3.13.7 to 3.13.8 twice more.

3.13.10 Add 100 μL NF water to each tube to re-suspend the bead mixtures, then transfer all of the solution (including beads) to a new 1.5 mL tube and centrifuge briefly.

3.13.11 Place the tube from step 3.13.10 onto a Magnetic Separation Rack for 2 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.13.12 Add 44 μL of NF water to each tube to re-suspend the bead mixtures, then transfer all of the solution (including beads) to a new 0.2 mL PCR tube.

3.14 Post-Capture PCR Amplification

3.14.1 Take out the MGIEasy Exome Capture Accessory Kit and prepare the Post-capture PCR amplification mixture on ice (see Table 25).

Table 25 Post- capture PCR Amplification Mixture

Components	Volume
Post-PCR Enzyme Mix	50 μL
PCR Primer Mix	6 μL
Total	56 μL

⚠ **Note: Post-PCR Enzyme Mix and PCR Primer Mix are included in the 'MGIEasy Exome Capture Accessory Kit.'**

3.14.2 Transfer 56 µL of the Post-capture PCR amplification mixture into each of the PCR tube(s) (including beads) from step 3.13.12. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.14.3 Place the PCR tube(s) from step 3.14.2 into the thermocycler and run the program described in Table 26.

Table 26 Post-capture PCR Amplification Reaction Conditions

Temperature	Time	Cycles
Heated lid	on	
95°C	3 min	1 cycle
98°C	20 s	
60°C	15 s	13 cycles
72°C	30 s	
72°C	10 min	1 cycle
4°C	Hold	

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

3.14.5 Place the tube(s) onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 100 µL supernatant from each tube to a new 1.5 mL tube.

3.15 Cleanup of Post-Capture PCR Product and Quantification

3.15.1 Take the DNA Clean Beads out of the refrigerator and allow 30 minutes to bring the beads to room temperature. Vortex and mix thoroughly before use.

3.15.2 Transfer 100 µL DNA Clean Beads to each tube from step 3.14.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that the liquid and beads are completely expelled from the pipette tip into the tube before proceeding.

3.15.3 Incubate at room temperature for 5 minutes.

3.15.4 Centrifuge briefly and place the tube(s) onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.15.5 Keep the tube(s) on the Magnetic Separation Rack and add 200 µL of freshly prepared 80% Ethanol to each tube to wash the beads and the walls of the tube. Incubate for 30 seconds and carefully remove and discard the supernatant.

3.15.6 Repeat step 3.15.5 once, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, place the tube back on to the

magnet rack, and remove the remaining liquid using a small volume pipette.

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

3.15.8 Remove the tube(s) from the Magnetic Separation Rack and add **32 µL** TE Buffer to each tube to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.15.9 Incubate at room temperature for 5 minutes.

3.15.10 Centrifuge briefly, then place the tube(s) back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **30 µL** supernatant from each tube to a different new 1.5 mL tube.

3.15.11 Quantify the purified post-capture PCR products with dsDNA Fluorescence Assay Kits such as the Qubit® dsDNA HS Assay Kit or the Quant-iT™ PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥ 1 pmol. Please refer to Appendix D to calculate the yield. For example, the desired yield for the fragmented DNA with a peak fragment size of 150 bp (Post-hybridization PCR products with a peak fragment size of 234 bp) should be ≥154 ng.

✔ **Stopping Point: After cleanup, purified PCR Products can be stored at -20°C.**

⚠ **Note: If the library will be delivered to a service lab for sequencing, please stop here. If the library will be sequenced in your lab, please go to step 3.16 to 3.20.**

3.16 Denaturation

⚠ **Note: Please read Appendix D carefully before you begin**

3.16.1 According to the PCR product size and Formula 1 in Appendix D, transfer 1 pmol of each PCR Product to a new 0.2 mL PCR Tube. Add TE Buffer for a total volume of 48 µL.

3.16.2 Place the PCR tube(s) from step 3.16.1 into the thermocycler and run the program in Table 27.

Table 27 Denaturation Reaction Conditions

Temperature	Time
Heated lid	On
95°C	3 min

3.16.3 After the reaction is complete, immediately place the PCR tube(s) on ice for 2 minutes, and centrifuge briefly.

3.17 Single Strand Circularization

3.17.1 Take out the MGIEasy Circularization Kit and prepare the single strand circularization reaction mixture in a new 0.2 mL PCR tube on ice (see Table 28).

Table 28 Single Strand Circularization Mixture

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

3.17.2 Transfer 12.1 μL of single strand circularization mixture into each PCR tube from step 3.16.3. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.17.3 Place the PCR tube(s) from step 3.17.2 into the thermocycler and run the program in Table 29:

Table 29 Single Strand DNA Circularization Reaction Conditions

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

3.17.4 After the reaction is complete, immediately place the tube(s) on ice for the next reaction.

3.18 Enzymatic Digestion

3.18.1 Prepare the following enzymatic digestion mixture (see Table 30) in a new 0.2 mL PCR tube on ice during the reaction in step 3.17.3.

Table 30 Enzymatic Digestion Mixture

Components	Volume
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

3.18.2 Transfer 4 μL of enzymatic digestion mixture into each PCR tube from step 3.17.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s).

3.18.3 Place the PCR tube(s) from step 3.18.2 into the thermocycler and run the following program in Table 31.

Table 31 Enzymatic Digestion Reaction Conditions

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

3.18.4 After the reaction is complete, centrifuge briefly to collect the solution at the bottom of the tube(s).

3.18.5 Immediately add 7.5 µL of Digestion Stop Buffer to each PCR tube. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube(s). Transfer all of the solution from each tube into a separate new 1.5 mL tube.

3.19 Cleanup of Enzymatic Digestion Product

⚠ **Note: Please read Appendix B carefully before you begin.**

3.19.1 Remove the DNA Clean Beads from the refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.

3.19.2 Transfer 170 µL of DNA Clean Beads to each tube containing Enzymatic Digestion product from step 3.18.5. Gently pipette at least 10 times to mix thoroughly. Ensure that the solution and beads are fully expelled from the tip into the tube.

3.19.3 Incubate at room temperature for 10 minutes.

3.19.4 Centrifuge briefly and place each 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant using a pipette.

3.19.5 Keep the 1.5 mL tube(s) on the Magnetic Separation Rack, add 500 µL of freshly prepared 80% Ethanol to each tube without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant.

3.19.6 Repeat step 3.19.5 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, place the tube(s) back on to the magnetic rack, then remove remaining liquid using a small volume pipette.

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

3.19.8 Remove the 1.5 mL tube(s) from the Magnetic Separation Rack and add **22 µL of** TE Buffer each tube to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly.

3.19.9 Incubate at room temperature for 10 minutes.

3.19.10 Centrifuge briefly. Place the 1.5 mL tube(s) back on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer **20 μL** supernatant from each tube to a separate new 1.5 mL tube. Be careful to not disturb the beads.

✔ **Stopping Point: Purified Enzymatic Digestion products can be stored at -20°C for one month.**

3.20 Quality Control of Enzymatic Digestion Product

Quantify the purified Enzymatic Digestion products using the Qubit® ssDNA Assay Kit. The final Enzymatic Digestion products (ng of ssDNA)/ input products of PCR (ng of dsDNA) should be ≥ 7%.

For example, if the input of PCR product used for the circularization has a main band peak around 234 bp by Bioanalyzer (corresponding to the insert fragment peak around 150 bp) is 154 ng total, the final yield after enzymatic digestion should be > 10.8 ng (154 x 0.07 = 10.8).

Appendix

Appendix A DNase I Digestion of RNA Sample

If there is DNA contamination in the RNA sample, you will need to perform a DNase I digestion (materials are not provided, see "Equipment and Materials Required but not Prepared"). DNase I digestion can cause some loss of RNA. The input amount of total RNA used in this step should be increased by 20% to 30%. For example, if the required input amount of RNA is 50 ng, the total RNA input should be 60-70 ng for the DNase I digestion. The protocol is shown below:

1. Transfer an appropriate amount of RNA sample to a RNase free 0.2 mL PCR tube and add NF water to the final volume of 42.5 μL. Prepare the DNase I digestion reaction mixture on ice (see Table 32):

Table 32 DNase I Digestion Reaction Mixture

Components	Volume
Total RNA	42.5 μL
DNase I	2.5 μL
10× DNase I Buffer	5 μL
Total	50 μL

2. Gently pipette the mixture and place the tube in the Thermocycler. Set up the following reaction conditions as listed in Table 33 below and start the reaction:

Table 33 The Reaction Conditions of DNase I Digestion

Temperature	Time
Heated lid (45°C)	on
37°C	20 min
4°C	∞

3. When the temperature reaches 4°C, remove and centrifuge the PCR tube briefly, then transfer the mixture to a new 1.5 mL tube, and proceed to the RNA clean up procedure below.

Note: Please read Appendix B carefully before you begin.

3.1 Remove RNAClean XP beads from 4°C storage and place them at room temperature for 30 min before use. Vortex thoroughly to mix well before use.

3.2 Transfer 90 μL RNAClean XP beads to the DNase I digestion product in Step 3. Gently pipette at least 10 times to mix thoroughly. Be careful to expel all of the liquid out of the tip and into the tube during the last mix.

3.3 Incubate the tube at room temperature for 5 minutes.

3.4 Centrifuge briefly. Place the tube on the magnetic rack for 2-5 minutes until the liquid clears. Carefully remove the supernatant with a pipette.

3.5 Keep the tube on the magnetic rack and add 200 μL freshly prepared 80% Ethanol with NF water to the tube without disturbing the beads. Incubate for 30 seconds and carefully remove and discard the supernatant.

3.6 Repeat step 3.5 for a total of two washes, remove all liquid from the tube without disrupting the beads. You may briefly centrifuge the tube to collect any remaining liquid to the bottom, place back on the magnet and then remove any remaining liquid using a small volume pipette tip.

3.7 Keep the tube on the magnetic rack with the lid open and allow beads to air dry until no wetness (reflectiveness) is observed. Do not let the beads over-dry or crack.

3.8 Remove the tube from the magnetic rack and add appropriate amount (12-13 μL) of NF water to elute the RNA. Pipette at least 10 times to mix thoroughly.

⚠ **Note: If the purified RNA sample needs to be quantified, elute the RNA by adding 13 μL of NF water, transfer 11 μL of supernatant to a new RNase free PCR tube and take 1μL of product for quantification using "Qubit™ RNA HS Assay Kit" (See "Equipment and Materials Required but not Provided"). If the quantification step is not performed, elute the RNA by adding 12 μL of NF water and transfer 10 μL of supernatant to a new RNase free PCR tube for RNA Fragmentation.**

3.9 Incubate the tube at room temperature for 5 minutes.

3.10 Briefly centrifuge. Place the tube on the magnetic rack for 2-5 minutes until liquid is clear. Carefully transfer the supernatant to a new RNase free PCR tube and proceed to the RNA Fragmentation.

Appendix B Magnetic Beads and Cleanup Procedures

For magnetic bead-based purification, we recommend using DNA Clean Beads supplied by MGI (MGI, Cat. No. 1000005278) or AMPure® XP (Agencourt, Cat. No. A63882). If you choose Magnetic Beads from other manufacturers, please optimize the cleanup conditions before getting started.

Before You Use

- To ensure capture efficiency of the Magnetic Beads, remove the beads from 4°C refrigerator storage, and equilibrate to room temperature for 30 minutes before use. Vortex and mix thoroughly immediately before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before each use.
- The volume of the beads (ratio beads: sample volume) determines the lower limit of fragment size that can be purified.

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add additional TE buffer to reach the designated volume before using the beads to purify. It ensures that the correct ratio for the beads is used.
- In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process should take approximately 2-3 minutes. Consider the different magnetic strength of your specific separation plate / rack and allow enough time for the solution to turn completely clear. If the supernatant is not completely clear, beads and product will be removed, and therefore lead to a reduction in sample eluted.
- Avoid contacting the beads with pipette tips when pipetting. 2-3 µL of fluids can be left in the tube to avoid contact. In the event of contact between the beads and the pipette tip, expel all of the solution and beads back into the tube and restart the separation process.
- Use freshly prepared 80% Ethanol (at room temperature) to wash the beads. Keep the tube on the Magnetic Separation Rack when washing with Ethanol. Do not shake or disturb the beads in any way.
- After the second bead wash with 80% Ethanol, try to remove all of the remaining Ethanol from within the tube. Centrifuge briefly to collect any remaining liquid at the bottom. Separate the beads magnetically and remove any remaining liquid by using a small volume pipette.
- After washing twice with 80% Ethanol, air-dry the beads at room temperature. Drying takes approximately 2-5 minutes depending on your laboratory environment. Watch closely until the pellet

appears sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.

- During the elution step, do not touch the beads with the pipette tip when removing the supernatant. Contamination of the sample with remaining beads may affect subsequent purification steps. Therefore, the total volume of TE buffer used for elution should be 2 μL more than the volume that is going to be eluted off. The remaining 2 μL should ensure no beads are removed with the purified sample.
- Pay attention when opening/ closing the tube lids on the Separation Rack. Strong vibrations may cause sample loss by spilling liquid or beads from the tubes. Secure the tubes before opening the lids.

Appendix C Combination Barcode Adapters Strategies

- This set includes one MGIEasy DNA Adapters-16 (Tube) Kit. This kit was developed to meet the requirements for multiplex sequencing. We selected the best Adapter combinations based on the principle of balanced base composition. However, the number of Barcode Adapters are not always continuous. For optimal performance, please carefully read instructions in Appendix C-1.
- MGI Adapters are double stranded. Please do not incubate above room temperature to avoid structural changes such as denaturation, which may affect performance.
- Before use, centrifuge to collect the Adapter liquid at the bottom of tube. Gently remove the cap to prevent spills and cross-contamination. Mix Adapters with a pipette before you use. Remember to close the cap immediately after use.
- Adapters from other MGI Library Prep Kits (number 501-596) are designed differently and are incompatible for mixed use. Mixed use will cause errors in barcode de-multiplexing during the data analysis procedures.
- Adapter quality as well as quantity directly affects the efficiency and quality of the final library. Please follow Table 34 to dilute the Adapter according to the input amounts of RNA sample used during processing.

Table 34 Recommended Adapter Input according to the Amount of total RNA

total RNA（ng）	MGI Adapter	MGI Adapter
	Dilution Ratio	Input after Dilution (μL)
51-100	5	5
10-50	10	5

- For other amounts of RNA sample input, please adjust the Adapter input appropriately.

C-1 MGIEasy DNA Adapters-16 (Tube) Kit Instructions

Based on the principles of balanced base composition, Adapters must be used in specific groups. Please follow the instructions below to use Adapters in proper combination:

2 sets of 4 Adapters: (01-04) and (13-16)

1 set of 8 Adapters: (97-104)

If the sequencing data output requirement is the same for all samples in one lane, please refer to Table 35 below to choose the appropriate barcode Adapter combinations.

Table 35 MGIEasy DNA Adapters-16 (Tube) Kit Instruction

Sample(s) /lane	Instructions (Example)
1	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix 4 Adapters with equal volumes, then add the mixture to the sample. Or 2. Take a set of 8 Adapters (e.g. 97-104), mix 8 Adapters with equal volumes, then add the mixture to the sample.
2	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), mix Adapters with equal volumes in pairs to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with equal volumes in groups of 4 to obtain 2 mixtures of equal volumes. Add 1 mixture to one sample. (e.g. Mix 97-100, then add to sample 1; Mix 101-104, then add to sample 2)
3	Requires at least 2 sets of Adapters: For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-2 and for sample 3.
4	Requires at least 1 set of Adapters: 1. Take a set of 4 Adapters (e.g. 01-04), add 1 Adapter to each sample in an equal volume. (e.g. Add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4, respectively.) Or 2. Take a set of 8 Adapters (97-104), mix Adapters with an equal volume in pairs to obtain 4 mixtures of equal volumes. Add 1 mixture to each sample. (e.g. Mix 97-98, 99-100, 101-102, 103-104, then add respectively to samples 1, 2, 3, 4.)
5	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for sample 5.
6	Requires at least 2 sets of Adapters: For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different Adapter sets for samples 1-4 and for samples 5-6.

7	Requires all 3 Adapter sets and follow these 3 steps: 1) For samples 1-4, use the method for (4 samples/lane) above (Use 1st Adapter set). 2) For samples 5-6, use the method for (2 samples/lane) above (Use 2nd Adapter set). 3) For sample 7, use the method for (1 sample/lane) above (Use 3rd Adapter set). You can add a single Adapter within the Adapter set. Or add the Adapter mix which is mixed from all Adapters within the Adapter set with an equal volume. Note that you should use different Adapter sets for samples 1-4, for samples 5-6 and for sample 7.
8	Requires at least 1 set of Adapters: 1. Take a set of 8 Adapters (97-104), respectively add 1 Adapter to each sample in an equal volume. Or 2. Take 2 sets of 4 Adapters (01-04 and 13-16), add 1 Adapter to each sample in an equal volume.

For situations in which the sequencing data output requirements are different between samples, any sample with a data output of more than 20% for each lane must use a separate set of Adapters.

For example, 9 samples are pooled into 1 lane, one of which requires 30% of the total data output. In this case, the other 8 samples may use Adapters (97-104), whereas the final sample must use a full Adapter set instead of using only a single Adapter (e.g. Adapter set (01-04) or (13-16)).

Appendix D Conversion between DNA Molecular Mass and Number of Moles

The formula below shows the calculation of the mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

$$\text{The mass (ng) corresponding to 1 pmol PCR Products} = \frac{\text{DNA Fragment Size (bp)}}{\text{1000 bp}} \times 660 \text{ ng}$$

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