iGeneTech
艾吉泰康

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For Illumina and MGI platform.
Generate WGS library.

Get in touch at:
www.igenetech.com/support
support@igenetech.com

IGT® DNA Library Prep Workflow with Enzymatic Fragmentation V3

Protocol

For use with:

- IGT® Enzyme Plus Library Prep Kit V3
- IGT® Adapter & Primer

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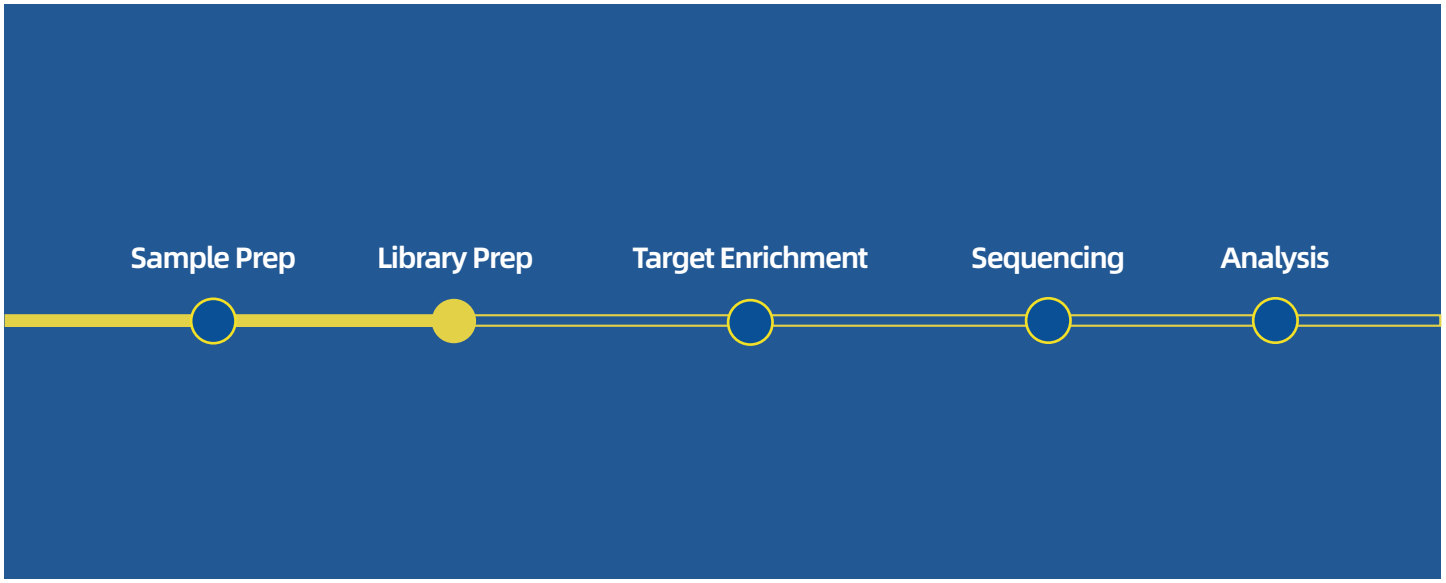

Version Notes

iGeneTech Bioscience (Jiaxing) Co., Ltd.

Level 1, Building 2, No. 371 Hongye Road, Dayun Town, Jiashan County, Jiaxing, Zhejiang 314113, China

Protocol Notes

The protocol provides an instruction for library construction using IGT® Enzyme Plus Library Prep Kit V3. Before you begin, please read this protocol carefully, and strictly follow the protocol for experiment.

Product Notes

The IGT® Enzyme Plus Library Prep Kit V3 is an universal DNA library preparation kit that uses enzymatic cleavage and generates sequencing libraries of Illumina and MGI platforms.

The kit adopts a one-step operation of DNA fragmentation, end-repair, and A-tailing with tunable enzymatic fragmentation time, making the insert size more controllable. The library preparation time is as short as 2.5 hours, which has the advantages of simple process, high library conversion rate, and wide sample compatibility. It is suitable for whole genome sequencing of 5 ng ~ 500 ng DNA and probe hybridization capture sequencing.

The kit is suitable for various sample types: genomic DNA (extracted from blood, blood cards, saliva, oral swabs, fresh or frozen tissues, cells, etc.), paraffin section sample DNA (FFPE DNA), etc.

History

Revision	Date	Description
A.0	September 2023	Initial release

Materials from iGeneTech

The complete library preparation process requires both a library preparation module and an adapter module, please select the proper iGeneTech adapter module according to your needs.

Library Preparation Module: IGT® Enzyme Plus Library Prep Kit V3

Screw Cap	Components	Amount		Storage
		16 rxn	96 rxn	
Red	Enhancer Buffer E v3	88 µL	540 µL	-20℃ ± 5℃
Yellow	Fragment & ERA Buffer v3	180 µL	1080 µL	
Yellow	Fragment & ERA Enzyme Mix v3	180 µL	1080 µL	
Blue	Adapter Ligation Buffer v3	540 µL	2*1584 µL	
Blue	Adapter Ligase v3	88 µL	540 µL	
White	PCR Master Mix	450 µL	2*1350 µL	

Adapter Module: IGT® Adapter & Primer

Please choose one of the following IGT® Adapter & Primer according to your needs.

IGT® Adapter & UDI Primer (for Illumina / MGI)

Screw Cap	Components	Amount		Storage
		96*1 rxn	96*10 rxn	
Blue	Adapter (15 µM)	540 µL	4*1350 µL	-20℃ ± 5℃
Plate or White	UDI Primer N (10 µM each)*	8 µL each	96*75 µL	

*N for Index Number

IGT® Adapter & Single-Indexed Primer (for MGI)

Screw Cap	Components	Amount	Storage
		96*1 rxn	
Blue	Adapter (15 µM, for MGI SI)	540 µL	-20℃ ± 5℃
White	TPE 1.0 Primer (20 µM, for MGI)	264 µL	
Plate	TPE 2.0 Indexed Primer N (20 µM, for MGI)*	4 µL each	

*N for Index Number.

IGT® UMI Adapter & UDI Primer (for Illumina / MGI)

Screw Cap	Components	Amount		Storage
		96*1 rxn	96*10 rxn	
Blue	Insert UMI Adapter (15 µM)	540 µL	4*1350 µL	-20℃ ± 5℃
Plate or White	UDI Primer N (10 µM each)*	8 µL each	96*75 µL	

*N for Index Number.

Materials Supplied by User

The following materials are recommended brands by iGeneTech. Alternative reagents and consumables that meet the experimental requirements can also be used. Please select the appropriate materials to start the experiment.

Reagents

No.	Item	Recommend	Supplier (Cat #)
1	Ethanol Absolute	Varies	Varies
2	Nuclease-Free Water	Nuclease-Free Water	Ambion (Cat # AM9930)
3	Pure Beads (Use either one)	Agencourt AMPure XP Kit	Beckman (Cat # A63880)
		IGT ® Pure Beads	iGeneTech (Cat # C80661)
4	Fragment Analyzer (Please select the corresponding kit according to the instrument for use)	Agilent DNA 1000 Kit	Agilent (Cat # 5067-1504)
		S2 Cartridge (Standard Cartridge)	BiOptic (Cat # C105101)
5	Nucleic Acid Quantification Assays	Qubit ™ dsDNA HS Assay Kit	Thermo Fisher (Cat # C47257)

Equipment

No.	Item	Recommend	Supplier (Cat #)
1	96-well Magnetic Stand	DynaMag-96 Side	Thermo Fisher (Cat # 12331D)
2	Fragment Analyzer	Agilent 2100 Bioanalyzer system	Agilent (Cat # G2939AA)
3	Nucleic Acid Quantification	Qubit 4.0 Fluorometer	Thermo Fisher (Cat # Q33226)
4	Thermal Mixer, 0.2 mL block	Eppendorf ThermoMixer® C	Eppendorf (Cat # 5382000015)
5	Vortex Mixer	Varies	Varies
6	Microcentrifuge	Varies	Varies
7	Ice Block	Varies	Varies
8	Thermal Cycler	Varies	Varies

Consumables

No.	Item	Recommend	Supplier (Cat #)
1	Qubit tubes (0.5 mL)	Varies	Varies
2	0.2 mL PCR tubes	Varies	Varies
3	0.2 mL 8-strip tubes	Varies	Varies
4	10 µL Pipette tips	Varies	Varies
5	200 µL Pipette tips	Varies	Varies

Workflow

	Library Preparation	Time
STEP 1	Sample Preparation and Pre-Treatment	10 min (Optional)
STEP 2	DNA Fragmentation, End-Repair, and A-Tailing	60 min
STEP 3	Adapter Ligation	20 min
STEP 4	Post-Ligation Purification	30 min
STEP 5	PCR Amplification	30 min
STEP 6	Post-PCR Purification	30 min

Before You Begin

For best results, please read the following precautions carefully before you begin.

- ☐ If the reagent amount used in each experiment is small, aliquot is recommended to avoid repeated freeze-thaw.
- ☐ Reagents stored at -20℃ should be thawed on ice and not at room temperature or under heating conditions.
- ☐ After the reagents are thawed, mix thoroughly and centrifuge briefly before use, ensuring that the reagent is at the bottom of the tube and has been mixed well.
- ☐ Buffer, primers, and other reagents used in the experiments are recommended to be mixed by vortexing or pipetting. For enzyme reagents, it is recommended to invert the tube several times and mix well, or to mix with a pipette to avoid violent shaking that affects enzyme activity.
- ☐ PCR tubes should be centrifuged briefly before opening to ensure the sample or reagent at the bottom of the tube and to avoid cross-contamination from liquid splashing during the opening process.
- ☐ Please preparing the reaction mixture on ice or ice block.
- ☐ IGT® Pure Beads or AMPure XP Beads are the recommended pure beads for purification in this protocol. Please assess the volume of pure beads used for purification via pre-experiments if not using the recommended ones.
- ☐ Please take out the pure beads from 4℃ freezer in advance, mix well and incubate at room temperature for 30 min before use.
- ☐ Please use freshly prepared 80% ethanol, and do not use long-term stored ethanol which will affect the performance due to ethanol volatilization.

The experiment can be started immediately if the conditions above are met.

STEP 1 Sample preparation and Pre-Treament

Experiment Notes

Reagents Required:

- Nuclease-Free Water
- IGT® Pure Beads (Optional)
- Enhancer Buffer E v3 (Optional)

Equipment Required:

- Magnetic Stand (Optional)

1.1 Sample requirement

1.1.1 Total Amount: This kit is suitable for library construction with a starting amount of 5 ng ~ 500 ng of DNA. To improve data quality, it is recommended that the DNA input amount is no less than 50 ng. Please use a fluorescence quantitative instrument (e.g. Qubit 4.0 Fluorometer) for DNA concentration determination, and measuring DNA concentration by Nanodrop is not recommended.

1.1.2 Quality & Purity: Agarose gel electrophoresis can be used to determine DNA integrity and the presence of protein residues. The ideal nanodrop A260/280 value for pure DNA samples is around 1.8 - 2.0, and A260/A230 should be over 2.0.

The low value of A260/280 (< 1.8) is generally due to protein contamination; if A260/A230 is less than 2.0, it may indicate the presence of guanidine.

1.2 Due to the sensitivity of fragmentase to EDTA, please confirm the DNA sample condition before starting the experiment and select the pre-treatment method accordingly:

1.2.1 If the DNA sample is purified and with no EDTA, no sample pre-treatment is required. Please add Nuclease-Free Water to a total volume of 40 µL and proceed to STEP 2 for library construction.

1.2.2 If the DNA sample contains EDTA or is unqualified for purity, e.g., high protein and guanidine residues, magnetic bead purification is recommended. Use 2X volume of IGT® Pure Beads and elute with 40 µL of Nuclease-Free Water before proceeding to STEP 2 for library preparation. Please refer to **Appendix 1** for specific operations.

1.2.3 If the DNA sample contains small amount of EDTA, when you choose not to purify it with pure beads, you can add Enhancer Buffer E v3 and Nuclease-Free Water to a total volume of 40 µL and then proceed to STEP 2 for library preparation.

Please use the following formula to calculate the EDTA final concentration in fragmentation reaction:

$$\text{EDTA Final Conc.} = \frac{\text{EDTA Conc. in Sample Solvent (mM)} \times \text{Sample DNA Volume (µL)}}{\text{Fragmentation Reaction Total Volume 60 (µL)}}$$

Or

$$\text{EDTA Final Conc.} = \frac{\text{EDTA Conc. in Sample Solvent (mM)} \times \text{Sample DNA Input Amount (ng)}}{\text{DNA Conc. (ng/µL)} \times \text{Fragmentation Reaction Total Volume 60 (µL)}}$$

Then based on the calculated EDTA final concentration, add Enhancer Buffer E v3 according to the following table:

EDTA Final Concentration	Enhancer Buffer E v3 Volume
< 0.1 mM	0 µL
0.1 mM	1 µL
0.2 mM	2 µL
0.3 mM	3 µL
0.4 mM	4 µL
0.5 mM	5 µL

When the solvent of DNA sample is Low TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA), there is no need to add the Enhancer Buffer E v3.

STEP 2 Fragmentation, End-Repair and A-Tailing

Experiment Notes

Reagents Required:

- Fragment & ERA Buffer v3
- Fragment & ERA Enzyme Mix v3

Equipment Required:

- Mini Centrifuge
- Vortex Mixer
- Thermal Cycler

2.1 Thaw Fragment & ERA Buffer v3 on ice. Mix thoroughly and centrifuge briefly. Place back on ice.

2.2 Thaw Fragment & ERA Enzyme Mix v3 on ice, mix by rotating (do not vortex to mix) and centrifuge briefly. Place back on ice.

2.3 Please set the thermal cycler program before the next step.

Temperature	Time
Heat Lid Temperature 85℃	
4℃	1 min
30℃	X min (refer to the table below)
65℃	20 min
4℃	Hold

The following enzyme digestion time is applicable to intact gDNA samples without degradation. For degraded gDNA, it is recommended to shorten the enzyme digestion time. For FFPE DNA, the enzyme digestion time is recommended to be 15 ~ 20 min, with the insert size around 150 ~ 250 bp.

Enzyme Digestion Time	Average Insert Size of Library	Average Insert Size of Sequencing Data
15 min	350 bp	280 bp
20 min	300 bp	265 bp
30 min	250 bp	230 bp
40 min	200 bp	200 bp
60 min	180 bp	180 bp

When sequencing is performed, the library will undergo circulization, amplification or other steps, which may have a preferernce on insert size. Therefore, the average insert size of sequencing data is shorter than that of electrophoresis result.

2.4 Prepare the reaction mixture on ice as below:

Component	Volume
DNA Sample from STEP 1	40 µL
Fragment & ERA Buffer v3	10 µL
Fragment & ERA Enzyme Mix v3	10 µL
Total Volume	**60 µL**

Fragmentase can cleave DNA at room temperature and exhibit weak enzymatic activity at 4℃ . Therefore, please prepare the reaction mixture on ice and shorten the contact time between fragmentase and DNA sample when outside the Thermal Cycler as possible.

If the sample number in a single experiment is large, to improve the consistency of the fragment size between samples, please add DNA samples to the tube first, and then quickly add the master mix of Fragment & ERA Buffer v3 and Fragment & ERA Enzyme Mix v3.

2.5 Mix gently by pipetting (do not vortex to mix) and centrifuge briefly.

2.6 Place the PCR tube on the thermal cycler and start the program.

2.7 Proceed to STEP 3 immediately when the program finished.

This is not a stop point, please proceed to STEP 3 immediately.

STEP 3 Adapter Ligation

Experiment Notes

Reagents Required:

- Adapter Ligation Buffer v3
- Adapter Ligase v3
- Adapter (15 μM)

Equipment Required:

- Mini Centrifuge
- Vortex Mixer
- Thermal Cycler

3.1 Thaw Adapter Ligation Buffer v3, Adapter (15 μM) on ice. Mix thoroughly and centrifuge briefly. Place back on ice.

3.2 Place Adapter Ligase v3 on ice. Mix well by rotating (do not vortex to mix) and centrifuge briefly. Place back on ice.

3.3 Dilute adapter (15 μM) to an appropriate concentration according to the table below.

DNA Input Amount	Adapter Concentration	Dilution Times
500 ng	15 μM	/
200 ng	15 μM	/
100 ng	15 μM	/
50 ng	15 μM	/
20 ng	7.5 μM	2
10 ng	3 μM	5
5 ng	1.5 μM	10

3.4 Prepare the reaction mixture on ice as below:

Component	Volume
Product from STEP 2	60 μL
Adapter (diluted)	5 μL
Adapter Ligation Buffer v3	30 μL
Adapter Ligase v3	5 μL
Total Volume	**100 μL**

To reduce adapter self-ligation, please add Adapter (diluted) to the reaction mixture first, and then add the Adapter Ligation Buffer v3 and Adapter Ligase v3.

If the sample number in a single experiment is large and a master mix is required, do not add the Adapter to the master mix. Please add the Adapter to the reaction mixture first and then add the mixture of Adapter Ligation Buffer v2 and Adapter Ligase v2 to reduce self-ligation.

3.5 Mix gently by pipetting (do not vortex to mix) and centrifuge briefly.

3.6 Place the PCR tube on the thermal cycler and start the program as below:

Temperature	Time
	Turn Off the Heat Lid
20℃	15 min
4℃	Hold

2.5 Proceed to STEP 4 immediately when the program finished.

This is not a stop point, please proceed to STEP 4 immediately.

STEP 4 Post-Ligation Purification

Experiment Notes

Reagents Required:

- Pure Beads (IGT® Pure Beads)
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water

Equipment Required:

- Mini Centrifuge
- Vortex Mixer
- Thermal Cycler

This step is described as one round of magnetic bead purification. If size selection required, please refer to **Appendix 2**.

4.1 Prepare 80% ethanol with absolute ethanol and Nuclease-Free Water in advance, and place at room temperature. Please use freshly prepared 80% ethanol for magnetic bead purification.

4.2 Vortex the pure beads to mix well, and equilibrate the beads to room temperature for 30 min.

4.3 Add X volume of pure beads to each ligated sample from STEP 3. Pipette or vortex to mix well, then incubate at room temperature for 5 min.

Adapter in Step 3	Pure Beads Volume: X μL
Adapter (for Illumina)	70 μL
Adapter (for MGI DI)	55 μL
Adapter (for MGI SI)	55 μL

The Illumina and MGI adapters differ in structure and thus the purification of post-ligation products require different volume of pure beads respectively.

4.4 Spin briefly on a mini centrifuge. Place the PCR tube containing beads on a magnetic stand and allow the beads to fully separate from the supernatant (approximately 3 min).

4.5 Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec.

4.6 Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant.

4.7 Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the beads to fully separate from the supernatant. Remove all traces of supernatant using a 10 μL pipette.

4.8 Keep the PCR tube on the magnetic stand at room temperature for 3 ~ 5 min to dry the bead pellet. Do not overdry the bead pellet.

4.9 Add 22 μL of Nuclease-Free Water. Remove the PCR tube from the magnetic stand. Vortex or pipette to mix well. Incubate at room temperature for 2 min.

4.10 Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 min and allow the pure beads to fully separate from the supernatant.

4.11 Transfer 20 μL of clear supernatant containing each library to a clean PCR tube. Please proceed to the STEP 5.

This is a safe stop point, and sample can be stored at -20℃ for up to one month.

STEP 5 PCR Amplification

Experiment Notes

Reagents required:

- UDI Primer or TPE Primer
- PCR Master Mix

Equipment required:

- Mini Centrifuge
- Vortex Mixer
- Thermal Cycler

5.1 Thaw PCR Master Mix on ice. Mix well by rotating and centrifuge briefly. Place back on ice.

5.2 Thaw UDI Primer or TPE Primer on ice. Mix thoroughly and centrifuge briefly. Place back on ice.

5.3 Prepare PCR reaction mixture on ice as below:

Component	Premixed Index Volume	Unpremixed Index Volume
Product from STEP 4	20 µL	20 µL
PCR Master Mix	25 µL	25 µL
UDI Primer N (10 µM each)	5 µL	/
TPE 1.0 Primer (20 µM, for MGI)	/	2.5 µL
TPE 2.0 Indexed Primer N (20 µM, for MGI)	/	2.5 µL
Total Amount	**50 µL**	**50 µL**

5.4 Mix well by gently pipetting (do not vortex to mix) and centrifuge briefly.

5.5 Place the PCR tube on the thermal cycler and start the program as below:

Temprture	Time	PCR C ycles
Heat Lid Temperature 105℃		
98℃	1 min	1
98℃	20 s	N cycles
60℃	30 s	
72℃	30 s	
72℃	2 min	1
4℃	Hold	1

DNA Input Amount	PCR Cycles (with yield of PCR product ≥ 1.5 µg)	
	Post-Ligation Purification	Post-Ligation Size Selection
500 ng	3 - 4	4 - 5
200 ng	4 - 5	5 - 6
100 ng	5 - 6	6 - 7
50 ng	6 - 7	7 - 8
20 ng	8 - 9	/
10 ng	9 - 10	/
5 ng	10 - 11	/

The above cycle numbers are applicable to intact and undegraded gDNA. For degraded gDNA and FFPE DNA, it is recommended to add 1 ~ 2 PCR cycles appropriately.

This is not a stop point, please proceed to STEP 6 immediately.

STEP 6 Post-Amplification Purification

Reagents Required:

- Pure Beads（IGT® Pure Beads）
- 80% Ethanol（freshly prepared）
- Nuclease-Free Water

Equipment Required:

- Mini Centrifuge
- Vortex Mixer
- Thermal Cycler

6.1 Freshly prepare 80% ethanol with absolute ethanol and Nuclease-Free Water, and place at room temperature for magnetic bead purification.

6.2 Vortex the magnetic bead to mix well, and equilibrate the pure beads to room temperature for 30 min.

6.3 Add 1X volume (50 μL) of pure beads to each amplified sample. Pipet or vortex to mix well. Incubate at room temperature for 5 min.

6.4 Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the pure beads to fully separate from the supernatant (approximately 3 min).

6.5 Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec.

6.6 Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant.

6.7 Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the pure beads to fully separate from the supernatant. Remove all traces of supernatant using a 10 μL pipette.

6.8 Keep the PCR tube on the magnetic stand at room temperature for 3 ~ 5 min to dry the bead pellet. Do not overdry the bead pellet.

6.9 Add 30 μL of Nuclease-Free Water. Remove the PCR tube from the magnetic stand. Vortex or pipet to mix well. Incubate at room temperature for 2 min.

6.10 Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 min and allow the pure beads to fully separate from the supernatant.

6.11 Transfer 28 μL of clear supernatant containing each library to a clean PCR tube.

6.12 Quantify each library using Qubit dsDNA High Sensitivity Assay Kit. Analyze the fragment size of each library using Agilent 2100 Bioanalyzer.

Experiment Notes

The experiment ends here !

Appendix 1 EDTA Removal

Reagents Required:

- Pure beads (IGT® Pure Beads)
- 80% Ethanol (Freshly prepared)
- Nuclease-Free Water

Equipment Required:

- Mini Centrifuge
- Vortex Mixer
- Thermal Cycler

1. Vortex the pure beads and equilibrate the beads to room temperature for 30 min.
2. Add 2X volume of pure beads to the DNA sample. For example, add 100 µL pure beads if the DNA sample is 50 µL.
3. Pipet or vortex to mix well. Incubate at room temperature for 5 min.
4. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the pure beads to fully separate from the supernatant (approximately 3 min).
5. Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec.
6. Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant.
7. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the pure beads to fully separate from the supernatant. Remove all traces of supernatant using a 10 µL pipette.
8. Keep the PCR tube on the magnetic stand at room temperature for 3 ~ 5 min to dry the bead pellet. Do not overdry the bead pellet.
9. Add 42 µL of Nuclease-Free Water. Remove the PCR tube from the magnetic stand. Vortex or pipet to mix well. Incubate at room temperature for 2 min.
10. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 min and allow the pure beads to fully separate from the supernatant.
11. Transfer 40 µL of clear supernatant containing each sample to a clean PCR tube.
12. Quantify each sample using Qubit dsDNA High Sensitivity Assay Kit.

Appendix 2 Post-Ligation Size Selection

Please choose to proceed this step carefully since it may cause more loss of original DNA fragments. This step is not recommended for severely degraded gDNA, FFPE DNA samples, or samples with DNA input less than 50 ng.

Please select the appropriate enzyme digestion time according to the expected insert size, so that the average insert size is consistent with what has been expected and the original DNA molecule rate could be improved.

Condition (Illumina)

Enzyme Digestion Time	Pure Beads Volume V1	Pure Beads Volume V2	Average Insert Size of Library	Average Insert Size of Sequencing Data
30 min	40 μL	20 μL	230 bp	200 bp
30 min	30 μL	20 μL	300 bp	250 bp
20 min	25 μL	20 μL	360 bp	300 bp
20 min	20 μL	15 μL	400 ~ 500 bp	350 ~ 400 bp

Condition (MGI)

Enzyme Digestion Time	Pure Beads Volume V1	Pure Beads Volume V2	Average Insert Size of Library	Average Insert Size of Sequencing Data
30 min	30 μL	20 μL	230 bp	200 bp
30 min	20 μL	20 μL	300 bp	250 bp
20 min	15 μL	20 μL	360 bp	300 bp
20 min	10 μL	15 μL	400 ~ 500 bp	350 ~ 400bp

1. Prepare 80% ethanol in advance with absolute ethanol and Nuclease-Free Water, and place at room temperature for later use. Please try to use freshly prepared 80% ethanol for magnetic bead purification.
2. Vortex the pure beads for 30 sec to mix well, and equilibrate the pure beads to room temperature for 30 min.
3. Add V1 volume of pure beads to each ligated product from STEP 3. Pipet or vortex to mix well. Incubate at room temperature for 5 min.
4. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the pure beads to fully separate from the supernatant (approximately 3 min).
5. Keep the PCR tube on a magnetic stand, carefully remove the supernatant into a new PCR tube.

Please keep the supernatant and discard the pure beads. 5 μL of liquid could be remained in the tube to avoid any bead carryover.

6. Add V2 volume of pure beads to the new PCR tube, pipet or vortex to mix well. Incubate at room temperature for 5 min.
7. Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec.
8. Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant.
9. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the pure beads to fully separate from the supernatant. Remove all traces of supernatant using a 10 μL pipette.
10. Keep the PCR tube on the magnetic stand at room temperature for 3 ~ 5 min to dry the bead pellet. Do not overdry the bead pellet.
11. Add 22 μL of Nuclease-Free Water. Remove the PCR tube from the magnetic stand. Vortex or pipet to mix well. Incubate at room temperature for 2 min.
12. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 min and allow the pure beads to fully separate from the supernatant.
13. Transfer 20 μL of clear supernatant containing each library to a clean PCR tube.

Note

Website：www.igenetech.com
E-mail：support@igenetech.com
Tel：+86-10-89146623

iGeneTech Bioscience Co., Ltd.
301 Building 9 Compound 8 - Area 1 Shengmingyuan Road, Zhongguancun Life Science Park, Changping District, 102206 Beijing, China

iGeneTech (Jiaxing) Bioscience Co., Ltd.
101, Building 2, No.371 Hongye Road, Dayun Town, Jiashan County, Jiaxing, 314113 Zhejiang, China

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