egSEQ® Enzymatic Library Preparation v3

Designed for Illumina® & MGI platforms

User Guide

Version 1.01
15th October, 2023

For use with:
- egSEQ Enzyme DNA Library Prep Kit v3.0
- egSEQ Adapter & UDI Primer

For Research Use Only.
Not for in-vitro diagnostic use unless otherwise specified.

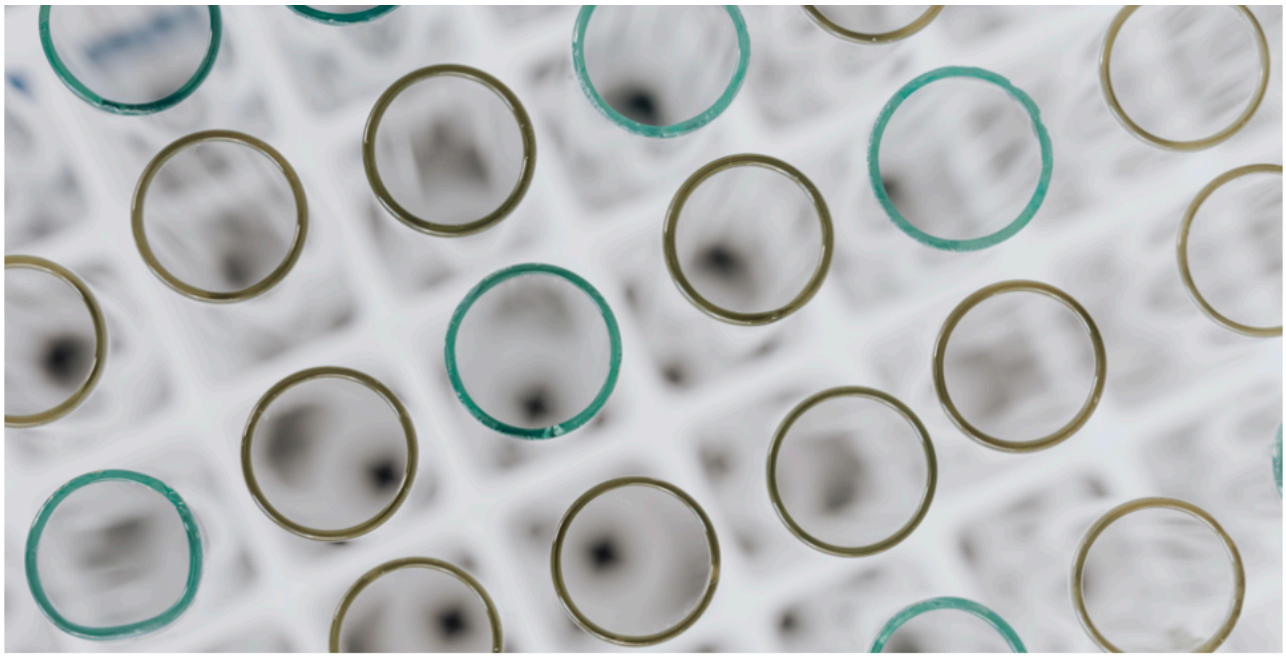
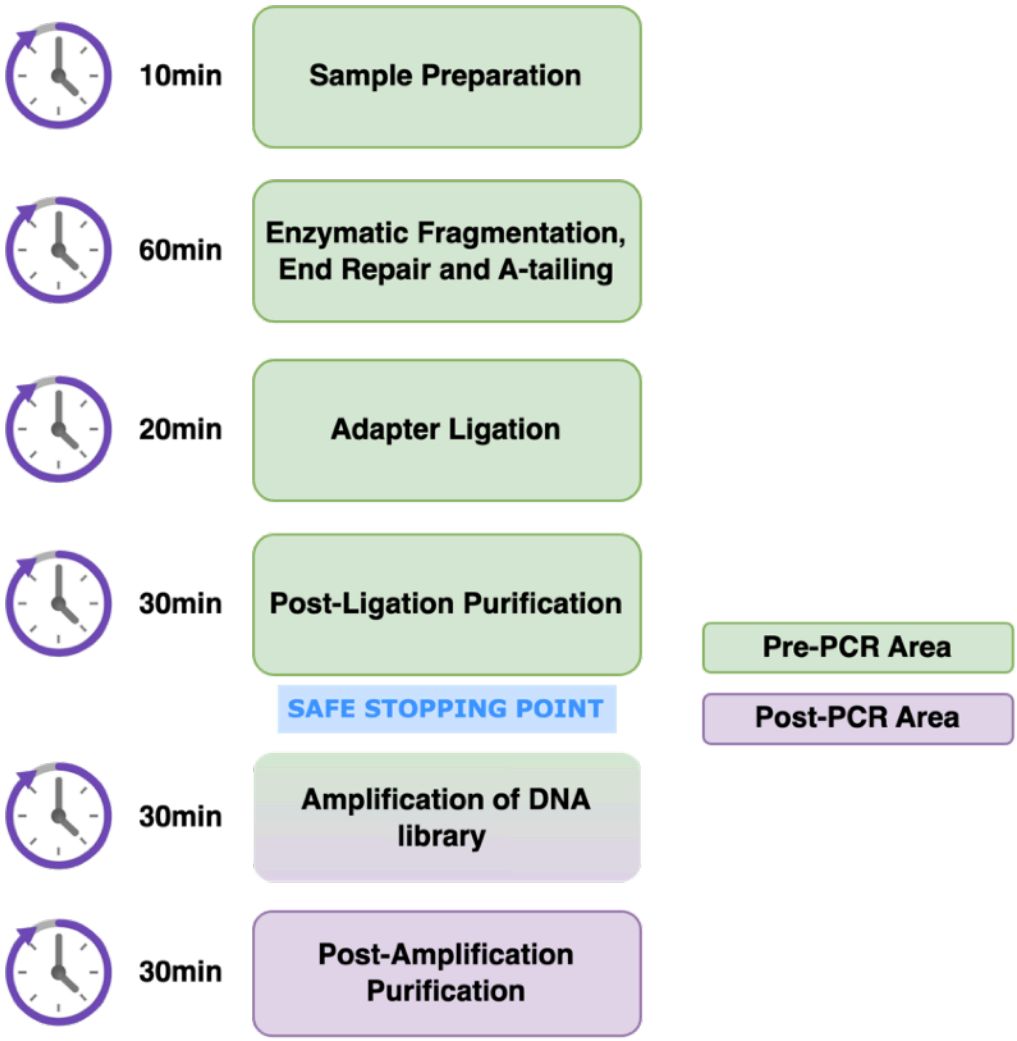


Table of Contents

Overview of Workflow

There are 6 steps in the workflow, outlined below with estimated time to completion for each step.



Materials Provided

The ingredients required for performing egSEQ enzymatic library preparation include:

- egSEQ Enzyme DNA Library Prep Kit v3.0
- egSEQ Adapter & UDI Primer

Library Preparation Module: egSEQ Enzyme DNA Library Prep Kit v3.0

Item	Screw Cap	Components	Storage	Volume	
				16 rxn	96 rxn
egSEQ Enzyme DNA Library Prep Kit v3.0	Red	Enhancer Buffer E v3	-20°C ± 5°C	88 µL	540 µL
	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: egSEQ Adapter & UDI Primer

Choose one of the following egSEQ Adapter & Primer according to your needs.

egSEQ Adapter & UDI Primer (for Illumina / MGI)

Item	Screw Cap	Components	Storage	Volume	
				96*1 rxn	96*10 rxn
egSEQ Adapter & UDI Primer 1-96 (< 384 indexes)	Blue	Adapter (15 µM)	-20°C ± 5°C	540 µL	4*1350 µL
	/	UDI Primer N (10 µM each)*		8 µL each	96*75 µL

* N for Index Number

egSEQ Adapter & Single-Indexed Primer (for MGI)

Item	Screw Cap	Components	Storage	Volume
				96*1 rxn
egSEQ Adapter & Single-Indexed Primer (for MGI)	Blue	Adapter (15 µM, for MGI SI)	-20°C ± 5°C	540 µL
	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

egSEQ UMI Adapter & UDI Primer (for Illumina / MGI)

Item	Screw Cap	Components	Storage	Volume	
				96*1 rxn	96*10 rxn
egSEQ UMI Adapter & UDI Primer (for Illumina / MGI)	Blue	Insert UMI Adapter (15 µM)	-20°C ± 5°C	540 µL	4*1350 µL
	Plate or White	UDI Primer N (10 µM each)*		8 µL each	96*75 µL

* N for Index Number

Other Required Materials

The materials below are recommended by Edinburgh Genetics. Please select appropriate materials based on experience and availability.

Reagents Required

Item	Recommendation	Supplier Catalogue #
Ethanol Absolute	-	-
Nuclease-Free Water	Nuclease-Free Water	Ambion (AM9930)
Magnetic Beads	egSEQ Pure Beads	Edinburgh Genetics (EG1200)
(Use either one)	Agencourt AMPure XP Kit	Beckman Coulter (A63880)
Fragment Analyzer	Agilent DNA 1000 Kit	Agilent (5067-1504)
Nucleic Acid Quantification Assays	Qubit® dsDNA HS Assay Kit	Thermo Fisher (C47257)

Equipment Required

Item	Recommendation	Supplier Catalogue #
96-well Magnetic Stand	DynaMag-96 Side	Thermo Fisher (12331D)
Fragment Analyzer	Agilent 2100 Bioanalyzer	Agilent (G2939AA)
Nucleic Acid Quantification	Qubit® 4.0 Fluorometer	Thermo Fisher (Q33238)
Thermal Mixer, 0.2 mL block	Eppendorf ThermoMixer® C	Eppendorf (5382000015)
Vortex Mixer	-	-
Microcentrifuge	-	-
Ice Block	-	-
Thermal Cycler	-	-

*Consumables Required**

Item	Recommendation	Supplier Catalogue #
Qubit tubes, 0.5 mL	Qubit® assay tubes	Thermo Fisher (Q32856)
PCR tubes, 0.2 mL	-	-
8-tubes strip, 0.2 mL	-	-
Pipette tips, 10 µL	-	-
Pipette tips, 200 µL	-	-

* References to PCR tube(s) in this guide also apply to plate(s). You may choose to use suitable PCR plate(s).

Before You Begin

Before beginning the experiment, please read the following notes carefully and where necessary confirm requirements are met:

- ❖ If the reagent amount used in each experiment is small, aliquoting is recommended to avoid repeated freeze-thaw cycles.

- ❖ Reagents stored at -20°C 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 avoid cross-contamination from liquid splashing during the opening process.

- ❖ Please prepare the reaction mixture on ice or ice block.

- ❖ egSEQ Pure Beads or AMPure XP Beads are the recommended magnetic beads for purification in this protocol. Please assess the volume of magnetic beads used for purification via pre-experiments if not using recommended brands.

- ❖ Please remove the magnetic beads from 4°C fridge 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 would affect the performance due to ethanol volatilisation.

Step 1: Sample Preparation

Reagents Required	Equipment Required
• Nuclease-Free Water • egSEQ Pure Beads (optional) • Enhancer Buffer E v3 (optional)	• Magnetic Stand (optional)

1.1. Sample Requirement

1.1.1. Total Amount: This kit is suitable for library preparation with a starting input of 5ng - 500ng of DNA. To improve data quality, it is recommended that the DNA input amount is no less than 50ng. Please use a fluorescence quantitative instrument (e.g. Qubit 4.0 Fluorometer) for determination of DNA concentration. 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.

1.2. Due to the sensitivity of fragmentase to EDTA, please confirm the DNA samples do not contain EDTA before starting the experiment and select the pre-treatment method accordingly:

1.2.1. If the DNA sample is purified and without 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 if any contamination is present, e.g., high protein and guanidine residues, magnetic bead purification is recommended. Use 2X volume of egSEQ 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 steps.

1.2.3. If the DNA sample contains small amounts of EDTA, if you choose not to purify it with magnetic 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. See information on the following page for how to prepare this.

Please use the following formula to calculate the final concentration of EDTA 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 & A-Tailing

Reagents Required	Equipment Required
• Fragment & ERA Buffer v3 • Fragment & ERA Enzyme Mix v3	• Thermal Cycler • Mini Centrifuge • Vortex Mixer

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

2.2. Place Fragment & ERA Enzyme Mix on ice. Mix well by rotating and centrifuge briefly. Place back on ice until further use.

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

Temperature	Time
Heat lid temperature 85°C	
4°C	1 minute
30°C	**X* minutes**
65°C	20 minutes
4°C	**Hold**

* The enzyme digestion times in the table below are applicable to intact gDNA samples. For FFPE DNA, the enzyme digestion time is recommended to be 15 - 20 min, with the DNA 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

Depending on the type of kit/number of cycles used, there may be a specific requirement for the final library size. Because the final library contains sequencing data insert, specific adapters and indexes, the sequencing data obtained will be shorter that the electrophoresis results (which will correspond to final library size).

2.4. Prepare the reaction mixture on ice as follows:

Component	Volume per Reaction
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°C . Therefore, prepare the reaction mixture on ice and minimise the contact time between fragmentase and DNA sample when outside the Thermal Cycler.

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 finishes.

Step 3: Adapter Ligation

Reagents Required:	Equipment Required:
• Adapter Ligation Buffer v3	• Thermal Cycler
• Adapter Ligase v3	• Mini Centrifuge
• Adapter (15 μM)	• Vortex mixer

3.1. Thaw the Adapter Ligation Buffer v3 and 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 the Adapter (15 μM) according to the table below.

DNA Input	Adapter Concentration	Dilute 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 indicated 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 is large and a master mix is required, do not add the Adapter to the master mix. Add the Adapter to the reaction mixture first and then add the mixture of Adapter Ligation Buffer v3 and Adapter Ligase v3 to reduce self-ligation.

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

3.6. Place the PCR tube on the thermal cycler, and start the program as indicated below.

Temperature	Time
Shut down heat lid	
20°C	15 minutes
4°C	**Hold**

3.7. Proceed to STEP 4 immediately when the program finishes.

Step 4: Post Ligation Purification

Reagents Required:	Equipment Required:
• egSEQ Pure Beads (or equivalent) • 80% Ethanol (freshly prepared) • Nuclease-Free Water	• Magnetic Stand • Vortex Mixer • Mini Centrifuge

This step describes one round of magnetic beads purification. If size selection is required, please follow the steps set out in **Appendix 2** before proceeding directly to **STEP 5: Amplification of DNA Library**.

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 magnetic beads to mix well, and equilibrate the beads to room temperature for 30 minutes.

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

Adapter in STEP 3	Magnetic 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 magnetic beads respectively.

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

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 seconds.

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 seconds. 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 minutes to dry the bead pellet. Do not over-dry 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 minutes.

4.10. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 minutes and allow the magnetic 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 STEP 5.

This is a safe stop point and samples can be stored at -20°C for up to one month.

Step 5: Amplification of DNA Library

Reagents Required:
- PCR Master Mix
- UDI Primer or TPE Primer

Equipment Required:
- Thermal Cycler
- Mini Centrifuge
- Vortex Mixer

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 well by rotating and centrifuge briefly. Place back on ice.

5.3. Prepare PCR reaction mixture on ice as indicated below.

Component	Premixed Index Volume	Unpremixed Index Volume
Sample 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 Volume	**50 µL**	**50 µL**

Ensure that the index number N of indexed primer is noted.

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

5.5. Place the PCR tube on the thermal cycler, and start the program below.

Temperature	Time	PCR Cycles
Heat lid temperature 105°C		
98°C	1 min	1
98°C	20 sec	N Cycles
60°C	30 sec	
72°C	30 sec	
72°C	2 min	1
4°C	**Hold**	**1**

DNA Input	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 intact gDNA. For degraded gDNA and FFPE DNA, it is recommended to add 1 - 2 PCR cycles appropriately.

5.6. When the PCR amplification finished, please proceed to STEP 6.

Step 6: Post Amplification Purification

Reagents Required:
- egSEQ Pure Beads (or equivalent)
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water
- Qubit dsDNA HS Assay (or equivalent)

Equipment Required:
- Magnetic stand
- Vortex mixer
- Mini Centrifuge
- Fragment Analyser
- Nucleic Acid Quantifier

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 beads to mix well, and equilibrate the beads to room temperature for 30 minutes.

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

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

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 seconds.

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 seconds. 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 magnetic 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 minutes to dry the bead pellet. Do not over-dry 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 minutes.

6.10. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 min and allow the magnetic 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 or equivalent. Analyse the fragment size of each library using Agilent 2100 Bioanalyzer or equivalent.

The experiment ends here!

Appendix 1: EDTA Removal

Reagents Required:
- egSEQ Beads (or equivalent)
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water

Equipment Required:
- Vortex Mixer
- Mini Centrifuge
- Magnetic Stand

1. Vortex the magnetic beads and equilibrate them to room temperature for 30 minutes.
2. Add 2X volume of magnetic beads to the DNA sample. For example, add 100 µL magnetic beads if the DNA sample is 50 µL.
3. Pipet or vortex to mix well. Incubate at room temperature for 5 minutes.
4. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the magnetic beads to fully separate from the supernatant (approximately 3 minutes).
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 seconds.
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 seconds. Remove and discard the clear supernatant.
7. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the magnetic 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 minutes to dry the bead pellet. Do not over-dry 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 minutes.
10. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 minutes and allow the magnetic 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 or equivalent.

Appendix 2: Post Ligation Size Selection

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

Select the appropriate enzyme digestion time according to the expected insert size, so that the average insert size is consistent with your expectations and the original DNA yield can be improved.

Condition (Illumina)

Enzyme Digestion Time	Magnetic Beads Volume V1	Magnetic 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	Magnetic Beads Volume V1	Magnetic 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 ~ 400 bp

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 magnetic beads for 30 seconds to mix well, and equilibrate the magnetic beads to room temperature for 30 minutes.
3. Add V1 volume of magnetic beads to each ligated product from STEP 3. Pipet or vortex to mix well. Incubate at room temperature for 5 minutes.

4. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the magnetic beads to fully separate from the supernatant (approximately 3 minutes).
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 magnetic beads. 5 µL of liquid can remain in the tube to avoid any bead carryover.

6. Add V2 volume of magnetic 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 seconds. Remove and discard the clear supernatant.
9. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the magnetic 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 minutes to dry the bead pellet. Do not over-dry 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 minutes.
12. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand for 2 minutes and allow the magnetic beads to fully separate from the supernatant.
13. Transfer 20 µL of clear supernatant containing each library to a clean PCR tube.
14. Please proceed to STEP 5.

This is a safe stop point and samples can be stored at -20°C for up to one month.

Technical Support

support@eggenetics.com
(44) 131 261 6686

eggenetics.com