WISGEN 欣基生物

xLIBPreP™ Enzymetics DNA Library Kit

Operating instruction

(Cat#NC002,Version4.5)

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Description

xLIBPreP™ Enzymetics DNA Library Kit is a universal, fast and efficient sequencing library construction kit compatible with illumina and MGI high-throughput sequencing platforms. The product is designed to fragment a large fragment of DNA by enzymatic fragment, with end repair and A-tail addition, without intermediate purification and with direct sequencing adapter ligation. The product is suitable for various sample types, including intact genomic DNA (Genomic DNA), paraffin sample DNA (FFPE DNA), immunoprecipitated DNA (ChIP DNA), etc. It is compatible with different DNA input amounts. The product provides a simple and fast library building process, which can be completed within 2 hours, greatly shortening the operation time, and the main experimental steps are as follows:

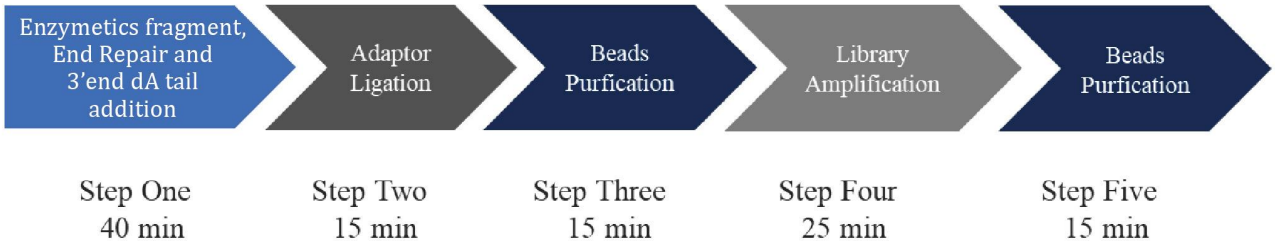

Figure 1.xLIBPreP™ Enzymetics DNA Library Construction Pipeline

Library Construction Workflow

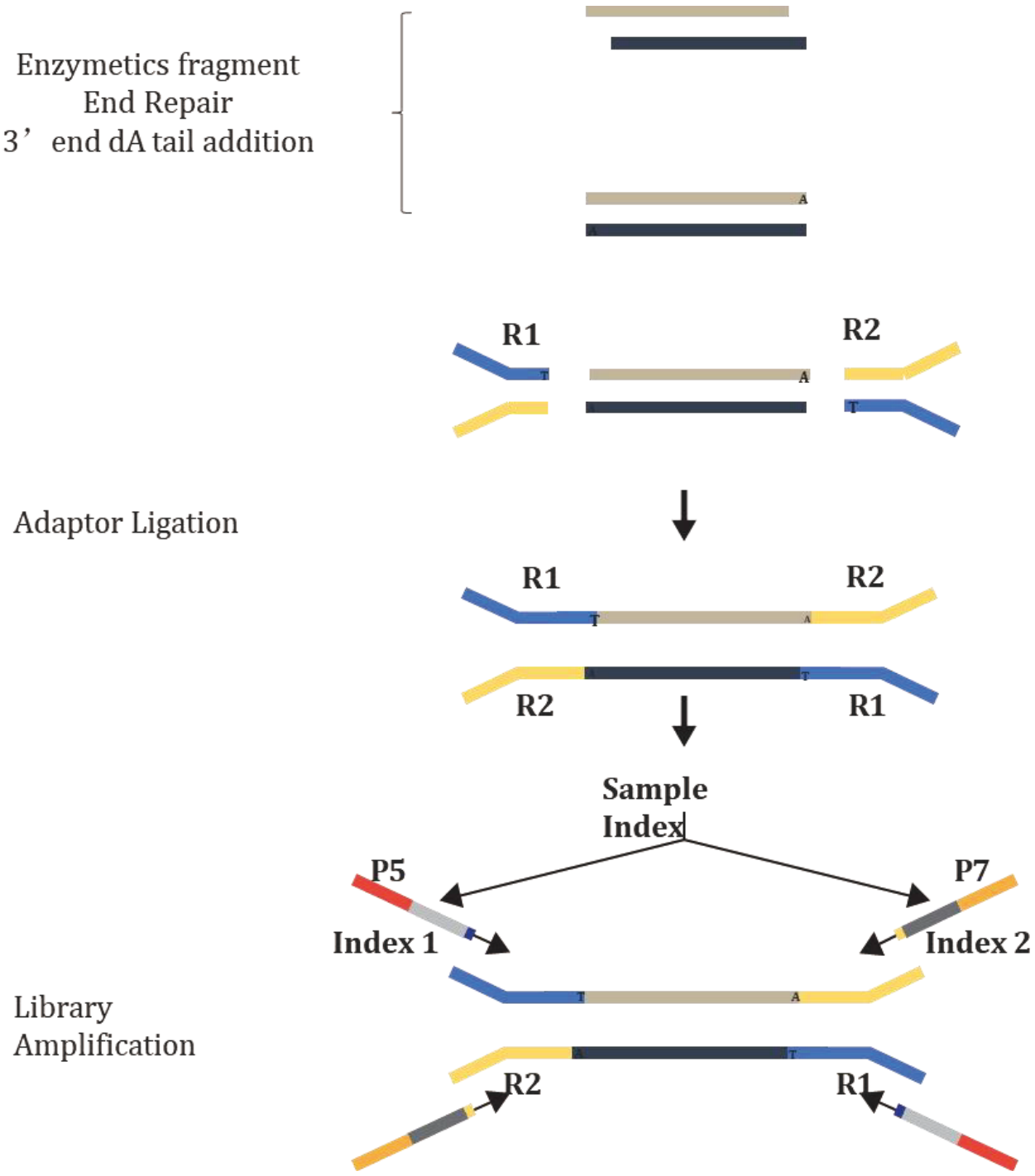

Figure 2. xLIBPreP™ Enzyzmetics DNA Library Kit Experimental Schematic

Reagent Detail

CatLog：NC002　　Specification: 24 rxns / 96 rxns

Product Component

Component	24rxns (NC002-024)	96rxns (NC002-096)	Expiration date
EFS Buffer	216 μL	864 μL	12 months
EFS Enzyme Mix	240 μL	960 μL	
Ligation Buffer	720 μL	2880 μL	
Ligase Enzyme	240 μL	960 μL	
2× HiFi Master Mix	600 μL	2400 μL	
1× TE	3 mL	6 mL	

Storage and delivery

-30 ～ -15 ℃ Storage , ≤0℃ delivery .

Application scope

This product is suitable for DNA library construction on illumina/MGI platform.

1. Sample type

   genomic DNA、FFPE DNA、ChIP DNA and so on.

2. DNA input quantity

   1~ 500 ng.

3. Application

   WGS

   WES or other target capture sequencing

Recommended for use with xCapSeq™ DNA Hyb&Washing Buffer Kit related products (Cat# HC00 series)

CHIP-seq

mNGS or tNGS

Methylation sequencing

Note

Please be sure to read this precaution before using this kit.

1. Please take care to avoid cross-contamination between nucleic acid samples and products during the operation.
2. Please use the tip and centrifuge tube without RNA enzyme or DNA enzyme for the experiment.
3. Before starting the experiment, please clean the operation table to ensure that there is no contamination of RNA enzymes and DNA.
4. Before performing library amplification, please make sure the PCR instrument has been tuned and is in stable condition.
5. Before using the purification beads, be sure to equilibrate them at room temperature for 30 min before use.
6. To ensure effective DNA fragmentation, DNA dilution in 1× TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) is recommended.
7. If the DNA is dissolved in 1× TE Buffer, the fragmentation reaction solution should be dilution with 1× TE Buffer, and if dilution with water, it will result in a severely small size library.
8. If the DNA has been dissolved in Low-EDTA solution such as Nuclease-Free Water, AE, etc. and the DNA concentration is low, 10× TE Buffer can be added to make the reaction in 1× TE Buffer.
9. 80% ethanol should be used on the same day to avoid volatilization of ethanol, which may cause library construction failure.
10. Different sequencing platforms have different adapter and primer set, you should choose the suitable adapter&primer set according to the platform and use the adapter&primer set according to the corresponding instructions.

11. All enzymes in the kit should be stored at -20°C immediately after use to avoid reducing the enzyme activity and affecting the library construction results.

12. Please read the instructions carefully before the experiment. If you need to pause the experiment or do not need to conduct downstream experiments immediately, you can follow the recommended steps in the instructions to store the experimental products at -20℃ and arrange subsequent experiments.

Consumables and Equipment

Consumables—WisGen

Item	Description	Catalog#	Stroge
xLIBPreP ™ Universal HIFI Amplication Kit (for illumina)	24 rxns	NC004-024	-30 ～ -15℃
	96 rxns	NC004-096	-30 ～ -15℃
xLIBPreP ™ Universal HIFI Amplication Kit （for MGI)	24 rxns	NC009-024	-30 ～ -15℃
	96 rxns	NC009-096	-30 ～ -15℃
xCapSeq™ SPRI Beads	50 ml	MB002-050	2~8℃
	450 ml	MB002-450	2~8℃
QuantiFast™ HS DNA Kit (for Qubit)	500 rxns	QC001-500	2~8℃
QuantiFast™ Tube (for Qubit)	500 tubes/pack	QC001-R-500	Room temp
xMag™ Magnet (1.5 mL *16 position)	1.5 mL *16 position	MR-B-16-3	Room temp
xMag™ Magnet (0.2 mL *16 position)	0.2 mL *16 position	MR-C-16-4	Room temp

Consumables—From other suppliers

Item	Supplier	Catalog#
Nuclease-Free Water	General laboratory supplier	Varies
Ethanol absolute (AR)	General laboratory supplier	Varies
0.1× TE buffer	General laboratory supplier	Varies
0.2 ml PCR MicroTubes (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies
0.5 ml MicroTubes (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies
1.5 ml MicroTubes (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies
10 μL Pipet Tips (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies
200 μL Pipet Tips (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies
1 mL Pipet Tips (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies
0.2 mL PCR Strip Tubes&Caps (Nuclease-Free)	Low adsorption，general laboratory supplier	Varies

Equipment

Item	Description
Thermal cycler	Bio-Rad C/S1000 or T100
Water bath or heating block	General laboratory supplier
Microcentrifuge	General laboratory supplier
Vortex mixer	General laboratory supplier
Fluorescence-based DNA quantitation system, such as Qubit® fluorometer (Thermo Fisher Scientific) for final quantitation of library	Various suppliers
Digital electrophoresis system	Agilent 2100 Electrophoresis Bioanalyzer® system (cat # G2939AA), Agilent 4200 TapeStation® System (cat # G2991BA), or equivalent

Tube Protocol

1. Enzymetic Fragment/End Repair/ 3'end dA addition

1.1 Reagent

1.1.1 Before starting the experiment, the concentration of the sample and whether the sample is dissolved in 1× TE Buffer needs to be clarified and the DNA quantified accurately.

Note: Determining the concentration of DNA is critical, especially when the sample amount is less than 100 ng. It is recommended to use the dye method for accurate quantification of DNA concentration. Also, please check which solvent the DNA is dissolved in, as different solvents may require slightly different handling.

Please refer to the following table for the recommended DNA input :

Application	Sample type	DNA input
WGS	Complex Genome	50~500 ng
Target Capture Seq	Complex Genome	10~500 ng
WGS/Target Capture Seq	FFPE DNA	≥10 ng
mNGS	Microbial Genome	1~500 ng
CHIP-Seq	ChIP DNA	≥1 ng

Note: The above is the recommended input amount of high quality DNA, when the DNA quality is poor, the DNA INPUT should be adjusted upward appropriately.

1.1.2 Melt the reagents of the kit on ice. EFS Enzyme Mix is mixed with finger flicks, not vortexed. The remaining reagents can be mixed by briefly vortexing.

1.2 Experimental steps

1.2.1 Take 0.2 mL PCR tubes and prepare the reaction according to the table below, operate on ice, and mix well by vortex shaking after all reagents have been added.

Component	Option 1 Volume / DNA Input amount	Option 2 Volume / DNA Input amount
EFS Enzyme Mix	10 μL	10 μL
EFS Buffer	9 μL	9 μL
DNA sample	X μL (200 ng)	X μL (200 ng)
1×TE Buffer*	41-X	/
10×TE Buffer*	/	4
Nuclease-Free Water	/	37-X
Total Volume	60 μL	60 μL

Note: For multiple reactions, please calculate the total volume of reagents required and add 10% to this volume, vortex and mix, then dispense into PCR tubes in order to avoid the problem of insufficient number of dispense reactions due to the loss of hanging walls during the solution transfer.

1.2.2 Set the thermal cycler (lid set at 90℃)reaction program according to the table below.

Step	Temp（℃)	Time
Fragmentation Time	32	3~30 min*
2	65	30 min
3	4	∞

* When DNA INPUT less than 50ng, The fragmentation time can be reduced by 1 to 3 min for FFPE samples, depending on the degree of DNA degradation, and by 1 to 5 min for FFPE samples.

1.2.3 The reference of fragmentation time.

Library size	Fragmentation Time	Optional Time
450 bp	5 min	3 ~ 10 min
300 bp	10 min	8 ~ 15 min
200 bp	15 min	10 ~ 20 min
150 bp	20 min	20 ~ 30 min

1.2.4 After complete vortex mixing of the reaction mix , the 0.2 mL PCR tubes were transiently centrifuged for 30 sec and immediately placed in the thermal cycler and the program was started.

Note: After centrifuging the PCR tube with mix , please take care not to generate air bubbles, which may affect the reaction.

1.2.5 After the reaction is completed, subsequent experiments should be performed as soon as possible, and it is recommended not to exceed 1 h at 4℃ Hold.

2. Adapter Ligation

2.1 Equilibrate the xCapSeq SPRI Beads or Beckman Ampure XP to room temperature (for at least 30 minutes). Suspend the beads thoroughly by vortexing.

2.2 This product does not contain DNA adapter , users can choose the appropriate adapter package kit according to the different sequencing platform.

CatLog	Product Name	Sequencing Platform
YG001	YWG™ Stubby Adapter & UDI Index Primer Set for illumina /GeneMind	Illumina、GeneMind
YG002	YWG™ UMI-Stubby Adapter & UDI Index Primer Set for illumina /GeneMind	Illumina、GeneMind
YG004	YWG™ DI Adapter & UDB Index Primer Set for MGI	MGI
YG005	YWG™ UMI-DI Adapter & UDB Index Primer Set for MGI	MGI
YG009	YWG™ SI Full Length Adapter Set for MGI	MGI

Note:The above recommended products are manufactured by WisGen Biosciences, if use products from other companies, please contact technical support (order@wisgen.cn) for guidance on their use.

2.3 The amount of the adapters used affects ligation efficiency and library yield. Please refer to table for the recommended adapter concentrations for different DNA inputs. If cfDNA input , don't dilution the adapter.

Inpμt DNA	Dilution Ratio	Working Concentration
1 μg ~ 50 ng	Undiluted	15 μM
49 ng ~25 ng	1:2	7.5 μM
24 ng ~10 ng	1:5	3 μM
9 ng ~ 5 ng	1:10	1.5 μM
< 5 ng	1:20	0.75 μM

Note: YWG Adapter are available in concentrations of 15 μmoL.

2.4 Mix the reagent below , adding the sequencing platform corresponding adapter.

Component	Volume
End Prep Reaction Mix	60 μL
Adapter*	3 μL
Nuclease-Free Water	7 μL
Total	70 μL

Note: To avoid self-linking of the Adapter, the Adapter should be added separately and not premixed with Ligation Buffer and Ligase Enzyme.

2.5 Mix the reagent below, vortexed well and blended on the ice.

Component	Volume
Adapter Reaction Mix	70 μL
Ligation Buffer	30 μL
Ligase Enzyme	10 μL
Total	110μL

Note: For multiple reactions, please calculate the total volume of reagents required and add 10% to this volume, vortex and mix, then dispense into PCR tubes in order to avoid the problem of insufficient number of dispense reactions due to the loss of hanging walls during the solution transfer.

2.6 Incubate the reaction mix in the thermal cycler (without set lid temperature), Start the program below.

Step	Temp	Time
1	22 ˚C	15 min
2	4 ˚C	∞

2.7 Purification of the ligation product is recommended using xCapSeq™ SPRI beads or Agencourt AMPure XP Beads. Add 0.8 X volume (88 μL) of magnetic beads to the reaction product for purification as follows:

2.7.1 Equilibrate the beads at room temperature for 30 min and vortex or shake to mix well before use.

Note: Be sure to equilibrate the beads at room temperature for 30 min before use, otherwise the DNA fragments size and DNA recovery will be affected.

2.7.2 After the ligation reaction program, incubate 0.2 mL PCR tube at room temperature for 5 min, then add 0.8× volume (88 μL) of magnetic beads to the ligation product for purification, and pipette or vortex for 30 sec.

2.7.3 Incubate at room temperature for 5 min, place the PCR tube on a magnetic rack for 2 min, and after the solution is completely clarified, carefully aspirate and discard the supernatant with a pipette, taking care not to aspirate the magnetic beads.

2.7.4 Keep the PCR tube on a magnetic rack, add 200 μL of 80% ethanol (ready-to-use), let stand for 30 sec (without blowing up the beads), and discard the supernatant.

2.7.5 Repeat step 2.7.4 once.

2.7.6 Remove the PCR tube containing the beads from the magnetic rack, centrifuge the tube instantaneously for 10 sec, place it on the magnetic rack, carefully remove the residual liquid with a small-volume pipette, and air dry at room temperature for 0.5-1min with the cap open until there are no visible water beads on the wall of the tube. Open the cap and dry at room temperature for 0.5~1min until there are no obvious water beads on the wall of the tube.

Note: Dry until there are no obvious water beads at the interface of the beads, do not over-dry to avoid cracking of the beads and lowering the DNA recovery.

2.7.7 Add 21 μL of Nuclease-Free Water or 0.1× TE Buffer to the PCR tube, gently pipette the beads until they are fully suspended, let them stand at room temperature for 5 min, then place the PCR tube on a magnetic rack for 2 min to allow the beads to fully adhere to the wall, and transfer the supernatant to a new PCR tube for subsequent PCR experiments.

Note: The product resulting from Adapter ligation after purification can be stable for approximately 1- 2 weeks at 4 °C/-20 °C.

3. PCR Amplification Reaction

3.1 Melt 2× HiFi Master Mix and PCR Primer Mix on ice and mix briefly.

3.2 Prepare the PCR reaction according to the table below, noting that this step should be performed on ice.

Component	体积
Purified ligation product	20 μL
2× HiFi Master Mix*	25 μL
PCR Primer Mix **	5 μL
Total Volume	50 μL

* 2× HiFi Master Mix should be added after other reagents or samples have been added to avoid a decrease in enzyme activity.

** PCR Primer Mix should be used with the corresponding Adapter. Different sequencing platforms use different adapter set, please refer to the corresponding adapter set instructions for different platform.

3.3 Mix the PCR reaction solution by gently pipetting 6 ~ 8 times or shaking and mixing for 10 sec.

Note: When preparing the mix , please keep the 0.2mL PCR tube on ice throughout the operation.

3.4 After transient centrifugation, the PCR tubes were placed in the thermal cycler.

3.5 Set the thermal cycler (set lid at 105℃)reaction program, and start.

Step	Temp(℃)	Time	Cycle Number
Pre-denaturation	98	1 min	1
Denaturation	98	10 sec	6 ~ 17*
Annealing	60	30 sec	
Extension	72	30 sec	
Extension	72	1 min	1
Hold	4	∞	1

* Please determine the number of PCR cycles based on the mass of DNA and the amount of sample to be uploaded. In general, for 100 ng, 10 ng, and 1 ng library starting DNA, 8, 14, and 17 cycles are required for PCR enrichment, respectively. If the DNA quality is relatively poor (e.g. FFPE DNA), it is recommended to add 1 to 4 cycles to the original.

* The specific parameters for the number of cycles can be found in the following table

DNA input	PCR cycles
1 ng	15 ~ 17
10 ng	12 ~ 14
25 ng	10 ~ 11
50 ng	9~ 10
100 ng	7 ~ 9
200 ng	6 ~ 8

3.6 When the PCR instrument temperature drops to 4°C, remove the PCR product from the thermal cycler to do purification. xCapSeq™ SPRI Beads or Agencourt AMPure XP Beads **(Refer to Appendix 1 if DNA size selection is required)** are recommended, as follows:

3.6.1 Equilibrate the magnetic beads at room temperature for 30 min and vortex or shake to mix before use.

Note: Be sure to equilibrate the beads at room temperature for 30 min before use, otherwise the sorted fragments and DNA recovery will be affected.

3.6.2 After the amplification is finished, incubate the PCR tube at room temperature for 5 min, then add 0.8× volume (40 μL) of magnetic beads to the amplification product and mix well by pipette for 30 sec.

3.6.3 Incubate at room temperature for 5 min, place the PCR tube on a magnetic rack for 2 min, and after the solution is completely clarified, pipette and discard the supernatant.

3.6.4 In the PCR tube, add 200 μL of 80% ethanol (ready-to-use) and let stand for 30 sec (without blowing up the magnetic beads) to remove the supernatant.

3.6.5 Repeat step 3.6.4 once.

3.6.6 Remove the PCR tube containing the beads from the magnetic rack, centrifuge the tube instantaneously for 10 sec, place it on the magnetic rack, carefully remove the residual supernatant with a small-volume pipette, and let it dry at room temperature for 0.5-1min with the cap open until there are no visible water beads on the tube wall.

Note: Dry until the bead interface is free of obvious water beads, do not over-dry to avoid cracking of the beads and lower DNA recovery.

3.6.7 Add 32 μL of Nuclease-Free Water or 0.1× TE Buffer to a 0.2 mL PCR tube and gently pipette the beads until fully suspended. After standing at room temperature for 5 min, place the reaction tube on a magnetic rack for 2 min to allow the beads to fully adhere to the wall and then transfer the supernatant to a new 0.2 mL PCR tube for subsequent experiments.

Appendix

1.DNA Size Select

1.1 Take out the xCapSeq™ SPRI Beads from 2-8C and keep the reagent at room temperature at least 30 min before use.

1.2 Mix the xCapSeq™ SPRI Beads thoroughly by vortex or turning upside down. Add xCapSeq™ SPRI Beads according to the 1st round of reaction volume in Table 2. Mix the xCapSeq™ SPRI Beads and sample thoroughly by pipette mixing 10 times.

1.3 Incubate the tube at room temperature for 5 min.

1.4 Place the tube onto the magnetic rack for about 2 min. After the solution is clarified, carefully transfer the supernatant to a new Nuclease-free tube.

1.5 Add xCapSeq™ SPRI Beads according to the 2nd round of reaction volume in Table 2 in the new tube, and mix the xCapSeq™ SPRI Beads and sample thoroughly by pipette mixing 10 times.

1.6 Incubate the tube at room temperature for 5 min.

1.7 Place the tube onto the magnetic rack for about 2 min. After the solution is clarified , carefully aspirate the supernatant and discard.

1.8 Keep the tube on magnetic rack . Dispense 200 µL of freshly prepared 80% ethanol into the tube and incubate for 30 sec at room temperature .

DO NOT re-suspend the beads ! Aspirate out the ethanol and discard.

1.9 Repeat step 8.

1.10 After removal of supernatant, centrifuged the tube for 10 sec, then keep the tube on the magnetic rack 30 sec, aspirate out the ethanol totally and uncap the tube to air - dry the magnetic beads for 30 ~ 60 sec.

1.11 Take out the tube from magnetic rack . Add an appropriate amount of Nuclease - free ddH2O to the tube and manually resuspend the beads by pipetting up and down 10 times . After incubated 5 min at room temperature , place the tube onto magnetic rack for about 2 min to separate beads from the solution , and carefully aspirate out the supernatant to a new Nuclease - free tube.

Table 2: Reference Conditions of DNA size selection

Bead Volume/DNA Volume	250bp -350bp	300bp -400bp	400bp -500bp	450bp -550bp	500bp -600bp	550bp -650bp
1st volume ratio	0.8x	0.7x	0.6x	0.55x	0.5x	0.45x
2nd volume ratio	0.2x	0.2x	0.2x	0.15x	0.15x	0.15x

Note："x" stand for sample DNA Volume .If DNA library insert size want 250 bp, sample DNA volume is 100μL, for the 1st volume ratio, xCapSeq™ SPRI Beads use 0.80×100 μL=80 μL； for the 2nd volume ratio, xCapSeq™ SPRI Beads 0.20×100 μL=20 μL.

2.Library Quality Control

2.1 Library size and yield of complete genomic DNA samples with different fragmentation times.

The DNA sample type was human peripheral blood DNA, 2 samples. The input volume was 200 ng, and the digestion time was 5 min, 10 min, 15 min, and 20 min at 32°C. The libraries were quantified using a Qubit®4.0 Fluorometer,and fragment size and peak shape analysis was performed using a Qsep100 Bio-fragment Analyzer (marker 1000bp).

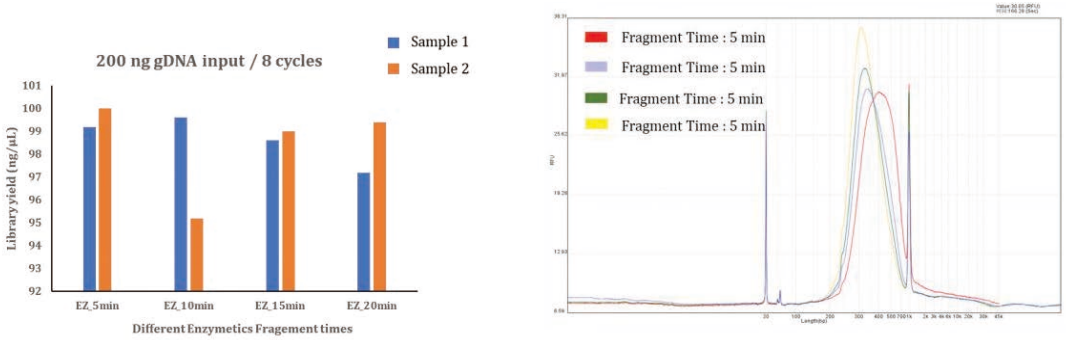

Fig. 1. Library size and yield of complete genomic DNA samples with different fragmentation times

2.2 Library size and yield of complete genomic DNA samples with different inputs.

The DNA sample type was human peripheral blood DNA, 3 samples. The input volume was 10 ng, 50 ng, and 200 ng, respectively, and the fragment time was 8 min at 32°C. The libraries were quantified using a Qubit®4.0 Fluorometer, and fragment sizes and peaks were analyzed using a Qsep100 Bio-fragment Analyzer (marker 1000bp).

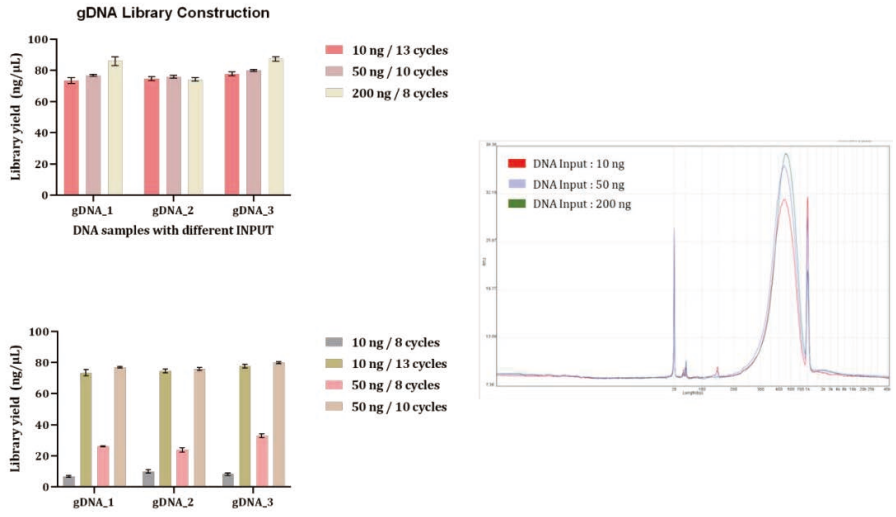

Fig. 2. Library size and yield of complete genomic DNA samples with different inputs

2.3 Size and yield of FFPE DNA library fragments with different degrees of degradation.

The DNA sample type was FFPE DNA, three samples with different degrees of degradation. 10 ng, 50 ng and 200 ng of FFPE A samples were input, and the fragment time was 5 min at 32°C. The libraries were quantified using Qubit®3.0 Fluorometer, and fragment size and peak pattern analysis were performed using Qsep100 Bio-fragment Analyzer (marker 1000bp). for fragment size and peak pattern analysis.

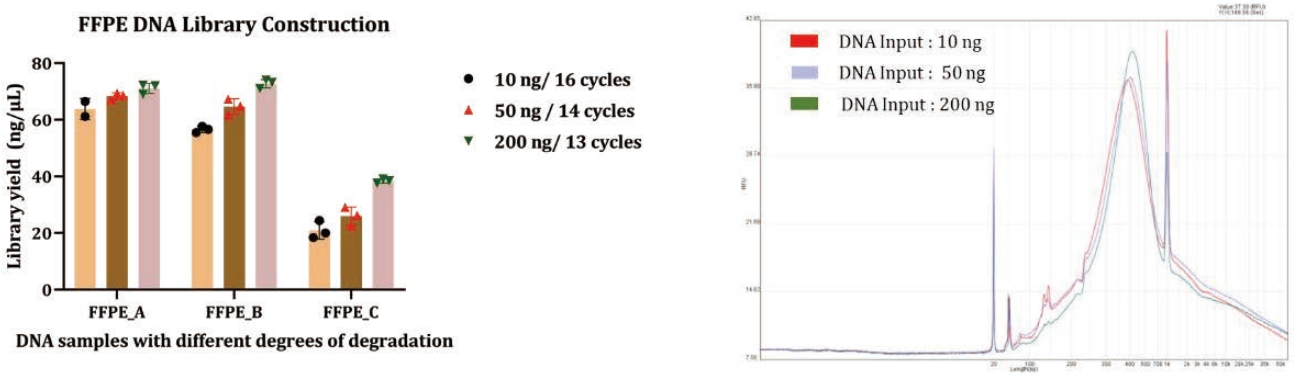

Figure 3. Size and yield of FFPE DNA library fragments with different degrees of degradation.

Sample Grading Criteria :

FFPE A: DNA main bands >2 kb with bright bands and no trailing bands;
FFPE B: DNA main bands below 2 kb with moderate dispersion;
FFPE C: DNA main bands distributed at 200-500 bp with dispersion.

2.4 Library QC Standard

2.4.1 Quantitative QC of the library was performed using a Qubit®3.0 Fluorometer with a library elution volume of 30 μL and a concentration of greater than 10 ng/μL.

2.4.2 The recommended library fragment size distribution is between 250 and 500 bp.

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