



ELLIS BIO

User Manual

SuperMethyl™ All-in-One Kit, 24 Reactions

End-to-End Bisulfite Sequencing Library Preparation

ALL-IN-24R

Version 2026.06

Questions? We're ready to help!

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Table of Contents

<i>Product Description</i>	<i>3</i>
<i>Specifications.....</i>	<i>3</i>
<i>Kit Components</i>	<i>4</i>
<i>Notes for Users</i>	<i>5</i>
<i>Experimental Protocol.....</i>	<i>8</i>
<i>Quality Control of the Final Libraries</i>	<i>17</i>

1. Product Description

SuperMethyl™ All-in-One Kit from Ellis Bio is a comprehensive, end-to-end solution for DNA methylation analysis, seamlessly integrating high-efficiency library preparation with **ultra-mild bisulfite conversion** for NGS applications. Designed to preserve DNA integrity while maximizing data quality, this all-in-one workflow enables accurate and sensitive methylation profiling from a wide range of sample types, **including gDNA, cfDNA, and FFPE-derived DNA**.

The integrated library preparation module uses methylated adapters and a robust amplification system to generate high-complexity libraries with **uniform GC coverage and enhanced CpG detection**, enabling more informative methylation data with fewer sequencing reads. The workflow is compatible with a broad DNA input range (as low as 100 pg) and supports both sonication- and enzymatic-fragmented DNA, ensuring flexibility across experimental designs.

Powered by proprietary Ultra-Mild Bisulfite chemistry, the **SuperMethyl™ Max Bisulfite Conversion Module** delivers highly efficient C-to-T conversion (99.8%) while minimizing DNA degradation and loss, critical for low-input and precious clinical samples. The streamlined workflow combines methylated adapter ligation, bisulfite conversion, cleanup, and library amplification into a compatible and optimized system, reducing handling steps and improving reproducibility.

With rapid processing, validated performance across sample types, and **all required components included**, the SuperMethyl™ All-in-One Kit delivers a reliable, efficient, and scalable solution for both research and translational methylation studies.

2. Specifications

Cat No.	ALL-IN-24R
Size	24 reactions

3. Kit Components

Number of tests per kit: 24 reactions

The SuperMethyl™ dsDNA Library Prep Module Includes:

Components No.	Components	Lib-Prep-24R	Storage
Lib-Prep-24R-A	Endprep Buffer ●	168 µL	-20 °C
Lib-Prep-24R -B	Endprep Enzyme ●	72 µL	-20 °C
Lib-Prep-24R -C	Ligation Enhancer ●	720 µL	-20 °C
Lib-Prep-24R -D	Rapid T4 DNA Ligase ●	240 µL	-20 °C
Lib-Prep-24R -E	Methyl Stubby Adapter ●	120 µL	-20 °C
Lib-Prep-24R -F	2 x Uracil + PCR Mix 2.0 ●	600 µL	-20 °C
Lib-Prep-24R -G	Methylated pUC19 ●	30 µL	-20 °C
Lib-Prep-24R -H	Unmethylated Lambda ●	30 µL	-20 °C

The SuperMethyl™ Max Bisulfite Conversion Module Includes:

Components No.	Component	MAX-24R-BEAD	Storage
MAX-24R-A	Conversion Reagent ●	2.4 mL	4 °C (recommended)
MAX-24R-B	Preparation Reagent ●	120 µL	Room temperature
MAX-24R-C	Enhancer A ●	120 µL	Room temperature
MAX-24R-D	Enhancer B ●	240 µL	4 °C (recommended)
MAX-24R-E	Binding Buffer	22 mL	Room temperature
MAX-24R-F	Wash Buffer*	7 mL*	Room temperature
MAX-24R-G	Desulphonation Buffer	6 mL	Room temperature
MAX-24R-H	Elution Buffer	1 mL	Room temperature
MAX-24R-I	Purification Beads ●	400 µL	Room temperature

* Wash Buffer requires the addition of 28 mL 100% ethanol (EtOH) before first use.

The DNA Selection Module Includes:

Components No.	Component	Select-24R	Storage
Select-24R-A	SuperMethyl™ DNA Selection Beads	5 mL	4 °C (recommended)

4. Notes for Users

Storage

1. SuperMethyl™ dsDNA Library Prep Module

We recommend storing all components of the Library Prep Module at -25 to -15 °C. The kit is stable for up to one year under these conditions.

2. SuperMethyl™ Max Bisulfite Conversion Module

We recommend storing **Conversion Reagent** ● and **Enhancer B** ● at 4 °C and avoid exposure to air and light. All other kit components can be stored at room temperature. The kit is stable for up to 12 months. Please refer to the product label for the expiration date.

3. SuperMethyl™ DNA Selection Beads

We recommend storing this product at 2 - 8 °C. The beads are stable for up to 18 months.

About the operation

1. Please operate with lab coats and disposable gloves for your safety.
2. Thaw components at room temperature. After thawing, mix thoroughly by vortexing, spin the tube briefly and place them on ice for later use.
3. When preparing the reaction solution in each step, it is recommended to use a pipette to blow and mix evenly or shake gently. Violent shaking may cause the library output to decrease.
4. In order to avoid cross contamination of samples, it is recommended to use a gun head with a filter element. Please replace the gun head when absorbing different samples.
5. It is recommended to perform each reaction step in a thermocycler with a heated lid. The thermocycler should be preheated to the set temperature before use.
6. Improper operations may very likely cause aerosol contamination, impacting the accuracy of result. Mandatory physical isolation of PCR reaction mixing regions and PCR product purification assay regions is recommended. Equipped with equipment such as specialized pipettes for library construction.
7. This product is for research use only.

About DNA fragmentation

1. This kit is compatible with DNA fragmented using either sonication or enzymatic methods.
2. If you use the enzymatic method to fragment gDNA, please use a suitable enzymatic kit. It is recommended to purify the DNA fragmented by the enzymatic method to remove the enzyme and buffer in the reaction system to avoid affecting the next step of the reaction.

Methylation controls

It is recommended to include appropriate positive and negative controls in the experiment. Spike-in controls such as unmethylated lambda DNA and methylated pUC19 DNA are useful options.

About Adapter Ligation

1. For the Illumina sequencing platform, the concentration of the Methyl Stubby Adapter provided by Ellis Bio is 5 μ M and can be used with SuperMethyl™ 384 CDI Primer for Illumina or SuperMethyl™ Stubby UDI Primer Kit for Illumina.
2. The concentration of the adapter directly affects the ligation efficiency and library yield. Excessive use of methylated adapter may produce more adapter dimer; Low dosage may affect the ligation efficiency and library yield. **Protocol Table 1** lists the recommended amount of adapter for different DNA inputs using this kit.

Protocol Table 1. The recommended Illumina™ adapter amount for different input DNA		
Input DNA	Adapter dilution multiple	Concentration
10 ng or below	5-fold dilution	1 μ M
25 ng	2.5-fold dilution	2 μ M
100 ng	No dilution	5 μ M
1000 ng	No dilution	5 μ M

About Library Conversion

1. This kit has been strictly verified and is compatible with the bisulfite conversion module: SuperMethyl™ Max Bisulfite Conversion Kit (MAX-24R-BEAD), SuperMethyl™ Fast Bisulfite Conversion Kit (FAST-50R-BEAD).
2. The recommended input range for bisulfite conversion is 0.1 ng-2000 ng.
3. The C-to-T conversion rate at all unmethylated cytosines in both CpG and non-CpG contexts of λ DNA exceeds 99.5%. The apparent methylation levels across CpG sites in fully methylated pUC19 control DNA are consistently greater than 95.0%, indicating effective preservation of methylated cytosines under the validated conditions.

About Library Amplification

Amplification cycles should be strictly controlled. Insufficient amplification may lead to low library yield; Over-amplification may introduce increased bias, errors, duplicated read, and chimeric products.

About Library Quality Analysis

1. The constructed libraries quality is generally analyzed by measuring the concentrations and size distributions.
2. Libraries concentrations can be measured by fluorescent-based methods such as Qubit or electrophoresis-based Agilent TapeStation system.
3. It is not recommended to use absorbance-based quantification methods such as NanoDrop.
4. It is recommended to use qPCR method for library quantification: fluorescent-based methods such as Qubit cannot differentiate the incomplete dsDNA structures (inserts with no adapter or with only one of the ends ligated with adapter) from the complete libraries. The qPCR method will only amplify and measure the complete libraries with both ends ligated with adapters (sequencable libraries), thus providing a more accurate measurement for loading.
5. The size distribution of libraries can be analyzed using Agilent Tapstation or other devices based on the principles of capillary electrophoresis or microfluidics.

Instructions

User-supplied materials

- 100% ethanol
- Nuclease-free H₂O
- 1.5 mL low-adhesion microcentrifuge tubes and PCR tubes.
- Microtube rotator
- Magnetic tube racks
- Library quality analysis: D1000 (or High Sensitivity D1000) ScreenTape Assay for TapeStation Systems or other equivalent products.

Workflow

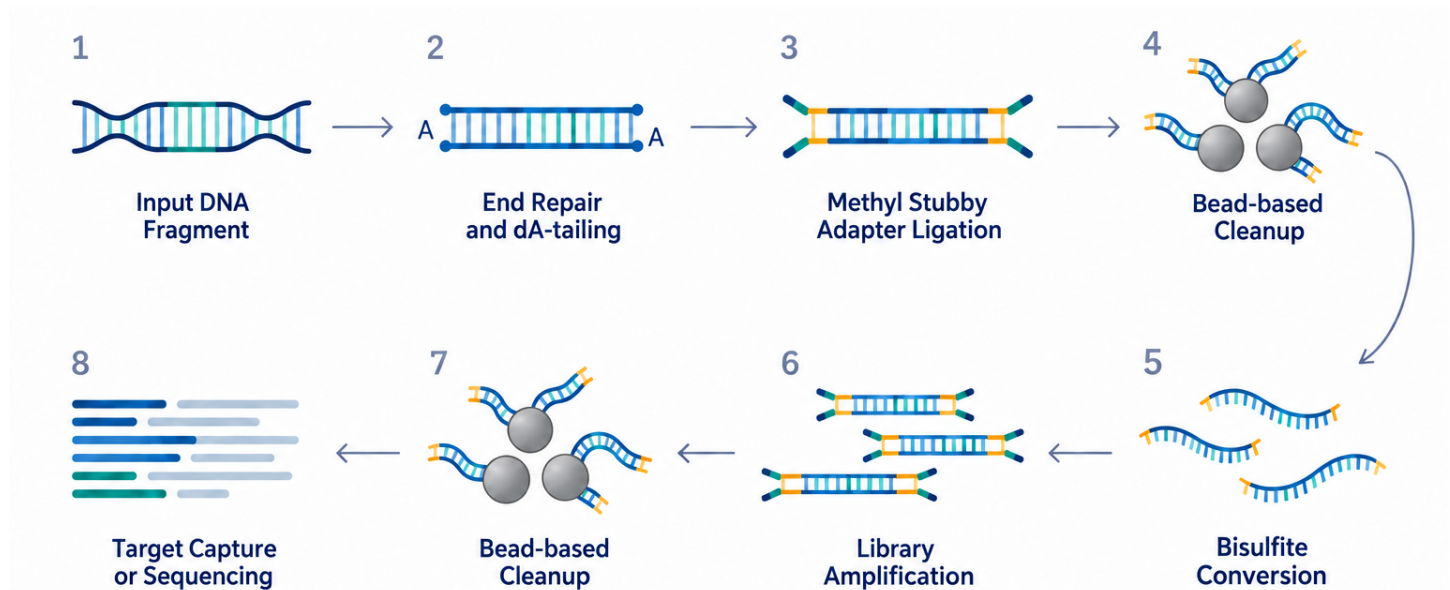


Figure 1. The workflow of SuperMethyl™ All-in-One Kit

5. Experimental Protocol

1. End Repair/dA-Tailing

This step performs DNA end-repair, 5'-end phosphorylated and dA-tailing in one reaction.¹

! ¹Note: Prior to this step, genomic DNA and FFPE DNA, along with unmethylated lambda DNA and methylated pUC19 DNA spike-in controls, should be fragmented. In contrast, cfDNA does not require fragmentation.

- 1.1. Thaw the reagents list in **Protocol Table 2**. Invert and mix thoroughly and place them on ice for later use.
- 1.2. Prepare the reactions on ice according to **Protocol Table 2**.

Protocol Table 2. DNA End Repair/ dA-Tailing Reaction Setup	
Components	Volume (μL)
Fragmented DNA	x
End prep Buffer	7
End prep Enzyme	3
ddH ₂ O	Up to 60

- 1.3. Gently mix by pipetting or shaking, Centrifuge briefly to get the solution down.
- 1.4. Place the tube in a thermocycler and set the program according to **Protocol Table 3** to perform DNA fragmentation, end-repair, and dA-tailing reaction.

Protocol Table 3. Thermocycler Program for DNA End Repair/ dA-Tailing	
Temperature	Time
Heat lid to 105 °C	on
4 °C	1 min
30 °C	30 min
72 °C	30 min
4 °C	Hold

2. Methyl stubby Adapter Ligation

This step enables the product from step 1 ligated with special Illumina™ methylated adapters.

- 2.1. Please refer to table 1 to dilute the adapter to appropriate concentration according to the amount of input DNA.
- 2.2. Thaw the reagents list in **Protocol Table 4**, invert and mix thoroughly, and place them on ice for later use.
- 2.3. Add the reagents list in **Protocol Table 4** to PCR tube.

Protocol Table 4. Methylated Adapter Ligation Reaction Setup ²	
Components	Volume (μL)
dA-tailed DNA	60
Methyl Stubby Adapter	5*
Ligation Enhancer	30**
Rapid T4 DNA Ligase	10***
ddH ₂ O	5
Total	110



²Note: *The original concentration of the Illumina™ Methyl Stubby adapter of Ellis Bio is 5 μM. Please dilute the adapter according to the input amount and make the volume of the adapter fixed at 5 μL.

**The Ligation Enhancer is viscous. Please mix thoroughly by inverting or vortexing and briefly centrifuge before use.

***To avoid self-ligation of the adapter, add Rapid T4 DNA Ligase last and make sure that the other components are mixed before adding.

- 2.4. Mix thoroughly by gently pipetting up and down, and spin down briefly to collect all liquid from the sides of the tube.
- 2.5. Incubate the sample in a preheated thermal cycler as shown in **Protocol Table 5** and perform the adapter connection reaction.

Protocol Table 5. Thermocycler Program of Adapter Ligation Reaction	
Temperature	Time
Heat lid to 105 °C	Off
20 °C	15 min
4 °C	Hold

3. Clean Up Post Ligation

This step is to purify and size-select the product in step 2 with magnetic beads. The purification can remove residue adapters, adapter dimers, or other unusable products.

- 3.1. Preparation: take the SuperMethyl™ DNA Selection Beads out of the fridge, and equilibrate at room temperature for at least 30 minutes. Prepare fresh 80% ethanol.
- 3.2. Thoroughly mix the beads by inverting or vertexing.
- 3.3. Add 88 µL SuperMethyl™ DNA Selection Beads (0.8x, Beads:DNA = 0.8:1) to the tube containing the adapter-ligated product and mix well, and incubate at room temperature for 5 minutes.
- 3.4. Centrifuge briefly to get the solution down and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully remove the liquid.
- 3.5. Keep the tube on the magnetic stand, directly add 200 µL freshly prepared 80% ethanol to the tube. Incubate at room temperature for 30 seconds and carefully remove the liquid.
- 3.6. Repeat step 3.5 again.
- 3.7. Keep the tube on the magnetic stand, open the cap and dry the beads until the beads are just cracked (no more than 5 minutes).
- 3.8. Take the tube off the magnetic stand for elution, and directly add 21 µL ddH₂O. Thoroughly mix by vortexing or pipetting up and down and incubate at room temperature for 5 minutes. Carefully transfer 20 µL supernatant to a new PCR tube without touching the beads.

Safe stopping point: Samples can be stored at 4 °C for up to 1 week and at -20 °C for up to 1 month.

4. Bisulfite Conversion of Adapter-Ligated DNA

4.1. Reagent Preparation (Optional)

- 4.1.1. **Prepare Wash Buffer:** Add 28 mL of 100% ethanol (EtOH) to the **Wash Buffer** before first use. Invert to mix thoroughly and ensure the bottle cap is tightly sealed to prevent ethanol evaporation, which could impact the effectiveness of the **Wash Buffer**.
- 4.1.2. **Prior to using materials in 2 mL screwcap vials:** For any kit component provided in a 2 mL screwcap vial (e.g., **Conversion Reagent** ●, **Purification Beads** ●, **Elution Buffer**, etc), briefly centrifuge the vial to remove material that may have pooled in the cap. Use a micropipette to gently resuspend the contents so that all settled material is evenly mixed before use.
- 4.1.3. **Conversion Reagent** ● **Notes:** Immediately prior to use, please inspect the **Conversion Reagent** ● for any signs of crystallization. Minor crystallization is normal, but if crystals are present, heat the vial at 55 °C or vortex until fully dissolved, then allow the reagent to equilibrate to room temperature before use. Failure to dissolve the crystals may significantly impair conversion efficiency.

4.2. Bisulfite Conversion

- 4.2.1. In a 0.2 mL nuclease-free PCR tube, pipette the volume to obtain 100 pg – 50 ng of input DNA. Add nuclease-free H₂O up to a total volume of 20 µL³.

 ³ Note: SuperMethyl™ Max Bisulfite kit is also compatible with larger DNA input volume (20-40 µL), please reach out to support@ellisbio.com for more information.

- 4.2.2. Add 2 µL of **Preparation Reagent** ● to the tube per **Protocol Table 6**. Mix thoroughly by vortexing or gentle pipetting. Briefly centrifuge to collect the liquid at the bottom of the PCR tube.

Protocol Table 6. Sample Preparation Reaction Setup	
Component	Volume
Input DNA	20 µL (50 pg - 100 ng)
Preparation Reagent ●	2 µL
Total Volume	22 µL

- 4.2.3. Place the capped PCR tube in a thermal cycler and incubate at 42 °C (with a 55 °C heated lid) for 20 minutes using the program shown in **Protocol Table 7**. After the incubation, transfer the sample immediately to ice for 2 minutes.

Protocol Table 7. Thermocycler Program of Sample Preparation

Step	Temperature	Time
1. Sample prep incubation	42 °C	20 min
2. Transfer immediately to ice for at least 2 minutes		

4.2.4. In a 1.5 mL nuclease-free microcentrifuge tube, prepare the **Conversion Mastermix**⁴ by adding components in the following order: first, add **Conversion Reagent** ●, then add **Enhancer A** ● and vortex immediately for 10 seconds. Next, add **Enhancer B** ● and vortex for an additional 10 seconds. Refer to **Protocol Table 8** for component volumes:

Protocol Table 8. Conversion Mastermix Preparation

Component	Volume
Conversion Reagent ⁴ ●	77 µL
Enhancer A ●	2 µL
Enhancer B ●	8 µL
Total volume	87 µL



⁴Note: See Step 4.1.3 above for an important note regarding crystallization of the Conversion Reagent. We recommend preparing the Conversion Master Mix after Step 4.2.3. **If the Conversion Reagent has been stored at 4 °C, incubate the vial at 55 °C for 20 minutes before use. We recommend using the Conversion Master Mix immediately after its preparation.** However, if the Conversion Master Mix is not used immediately after its preparation, incubate it at 55 °C to minimize the risk of precipitation prior to Step 4.2.5.

4.2.5. Prepare the bisulfite conversion reaction according to **Protocol Table 9**. After adding the **Conversion Master Mix**⁵ to the **Post-sample Preparation DNA** for each sample, **immediately mix thoroughly by pipetting 10 times**. Briefly centrifuge to collect liquid at the bottom of the PCR tubes. If the sample volume exceeds the thermocycler settings, please split the sample into additional PCR tubes as needed.

Protocol Table 9. Bisulfite Conversion Reaction Setup

Component	Volume
Post-sample preparation DNA	22 µL
Conversion Master Mix	87 µL
Total volume	109 µL



⁵Note: Check the Conversion Master Mix for visible precipitation before use. If precipitation is observed, prepare a fresh Conversion Master Mix. **Following addition of the Conversion Master Mix to the post-sample preparation DNA, mix by pipetting up and down 10 times to ensure homogeneity immediately. For plate-based conversion incubations, vortex mixing is not permitted.** Vortexing can cause the master mix to come into contact with the sealing film, potentially resulting in substantial precipitate formation. The resulting precipitation may negatively impact conversion efficiency and compromise downstream cleanup and purification performance.

4.2.6. Place the capped PCR tubes in a thermal cycler and incubate at 55 °C (with a 75 °C heated lid) using the following thermocycler program in **Protocol Table 10**.

Protocol Table 10. Thermocycler Program of Bisulfite Conversion		
Step	Temperature	Time
1. Bisulfite conversion	55 °C	90 mins
2. Hold ⁶	20 °C	< 5 mins
3. Proceed to next step immediately⁷		

 ⁶Note: This step is optional; **we recommend proceeding directly to the next step**. Please do not place the sample on ice or store it at 4 °C, as this may lead to white crystallization formation, which can reduce the DNA recovery rate.

 ⁷Note: **After conversion, proceed immediately to Step 4.3.2. Do not vortex the converted reaction before adding it to the Binding Buffer and Purification Beads, as this may cause the solution to become cloudy or form precipitates, which can negatively impact the efficiency of the cleanup and purification process.**

4.3. Purification of Bisulfite-converted DNA

4.3.1. Add 800 µL of **Binding Buffer** and 15 µL of **Purification Beads**⁸  to a 1.5 mL low-adhesion microcentrifuge tube.

 ⁸Note: Purification Beads settle quickly, pipette or vortex thoroughly to keep them well-suspended. When working with multiple samples manually, we recommend vortexing the Purification Bead and Binding Buffer solution frequently (for example, every 4 samples) to maintain consistent suspension.

4.3.2. Transfer the bisulfite converted reaction mixtures from the PCR tubes (from Step 4.2.6) into the 1.5 mL microcentrifuge tube containing the **Binding Buffer** and **Purification Beads**  (Step 4.3.1). Mix thoroughly by pipetting up and down or by gently vortexing at low speed for 10 seconds. Incubate at room temperature (15 - 25 °C) for 10 minutes with continuous rotation. **A microtube rotator or mixer is strongly recommended for this incubation step.**

4.3.3. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads at the bottom, then place it on a magnetic stand for 5 minutes or until beads pellet has collected on magnet and the supernatant is clear. While the microcentrifuge tube on the magnetic stand, remove and discard the supernatant⁹.

 ⁹Note: If some Purification Beads adhere to the non-magnetic side of the tube, remove the supernatant slowly, allowing beads to migrate to the magnetic side as the liquid volume decreases.

4.3.4. Remove the microcentrifuge tube from the magnetic stand for this step and each subsequent buffer addition.

4.3.5. Add 400 µL of **Wash Buffer** (ensure 100% ethanol was added in Step 4.1.1 before first use) to the beads and resuspend by pipetting or by vortexing the microcentrifuge tube at low speed for 10 seconds.

- 4.3.6. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes or until the supernatant is clear. Carefully remove and discard the supernatant.
- 4.3.7. Add 200 μ L of **Desulphonation Buffer** to the beads. Resuspend by pipetting or by vortexing the microcentrifuge tube at low speed for 10 seconds.
- 4.3.8. Incubate at room temperature for 10 minutes with continuous rotation. **A microtube rotator is strongly recommended for this incubation step.**
- 4.3.9. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes or until the supernatant is clear. Carefully remove and discard the supernatant.
- 4.3.10. Add 400 μ L of **Wash Buffer** to the beads. Resuspend by pipetting or by vortexing the microcentrifuge tube at low speed for 10 seconds.
- 4.3.11. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes or until the supernatant is clear. Carefully remove and discard the supernatant.
- 4.3.12. Repeat Steps 4.3.10 and 4.3.11. After the final wash and discarding the supernatant, use a 10 μ L tip to remove as much **Wash Buffer** as possible. This will help ensure efficient drying of the purification beads.
- 4.3.13. Place the **uncapped microcentrifuge tubes** on a heating element to **completely dry the Purification Beads¹⁰** at 55 °C for 5 - 10 minutes.



¹⁰Note: **Ensure the Purification Beads are completely dry before proceeding to the elution step.** An example photo of dried beads is shown in the Supplementary Material. If needed, extending the heating time may improve recovery of bisulfite-converted DNA. If the heating element has a lid, please keep the heating element lid open during this step. Because heating efficiency may vary between heating blocks, we recommend optimizing the heating time to achieve optimal recovery.

4.4. Elution of Bisulfite-converted DNA

- 4.4.1. Add 27 μ L of **Elution Buffer¹¹** directly to the dried beads and resuspend by pipetting or vortexing the microcentrifuge tube at low speed for 10 seconds.



¹¹Note: The volume of Elution Buffer can be adjusted according to the requirements of downstream applications. Varying the elution volume may affect elution efficiency: **approximately 5 μ L of elution buffer typically remains associated with the magnetic beads after separation.**

4.4.2. Incubate the elution at 55°C for 4 minutes **with the cap closed**, mixing at 1000 RPM. Then place the microcentrifuge tube on a magnetic stand for 3 minutes or until the supernatant is clear.

4.4.3. Carefully transfer the supernatant (eluate) to a clean microcentrifuge tube or PCR tube for downstream amplifications.

Safe stopping point: The eluate, containing bisulfite-converted DNA, is immediately ready for downstream applications such as PCR analysis or next-generation sequencing. For storage, keep the eluate at -20 °C for short-term use or at -80 °C for long-term use.

5. Library Amplification

This step will perform PCR amplification and enrichment on the converted products.

5.1. Thaw the reagents list in table 6, invert and mix thoroughly, and place them on ice for later use.

5.2. Assemble the following reaction in a sterilized PCR tube in **Protocol Table 11**.

Protocol Table 11. PCR Amplification Reaction Setup ¹²	
Component	Volume (μL)
Converted Adapter-Ligated-DNA	20
2 x Uracil + PCR Mix 2.0	25
i5 index Primer*	2.5
i7 index Primer*	2.5
Total	50



¹²Note: *This kit uses index-free adapters. You can use the index primers for PCR amplification.

5.3. Gently mix by pipetting or shaking, and centrifuge briefly to get the solution down.

5.4. Put the tube into a thermocycler and set up the program according to **Protocol Table 12** to start the amplification.

Protocol Table 12. Thermocycler Program of PCR Amplification		
Temperature	Time	Cycle
Heat lid to 105 °C	On	-
98 °C	1 min	1
98 °C	10 sec	4-14
60 °C	30 sec	
72 °C	1 min	
72 °C	5 min	1
4 °C	Hold	-

6. Post-Amplification Clean-up/Size Selection

- 6.1. Preparation: Take out the SuperMethyl™ DNA Selection Beads from the 4°C and equilibrate at room temperature for at least 30 minutes. Prepare fresh 80% ethanol.
- 6.2. Vortex or fully invert the magnetic beads.
- 6.3. Add 45 µL SuperMethyl™ DNA Selection Beads (0.9X, Beads:DNA = 0.9:1) to amplification product and mix well on a vortex mixer or by pipetting up and down at least 10 times, and incubate at room temperature for 5 min.
- 6.4. Spin the PCR tube briefly and place it on a magnetic stand to separate the magnetic beads and liquid. After the solution is clear (about 5 minutes), carefully discard the supernatant.
- 6.5. Keep the PCR tube on the magnetic stand, add 200 µL of freshly prepared 80% ethanol to the tube, incubate at room temperature for 30 sec, carefully remove the supernatant.
- 6.6. Repeat step 5 once for a total of 2 washing steps.
- 6.7. Always keep the PCR tube on the magnetic stand, completely remove the residual ethanol, and air-dry beads for 5 minutes while the tube is on the magnetic stand with the lid open (no more than 5 minutes).
- 6.8. Remove the PCR tube from the magnetic stand, Elute DNA target from the beads with 32 µL ddH₂O. Mix well on a vortex mixer or by pipetting up and down, and incubate for 5 minutes at room temperature. Briefly spin the tube and put the tube back on the magnetic stand until the solution is clear, carefully transfer 30 µL of supernatant to a new PCR tube for library quantification and quality analysis.

7. Quality Control of the Final Libraries

The quality of the constructed library is generally evaluated by measuring the library concentration and DNA fragments size distribution.

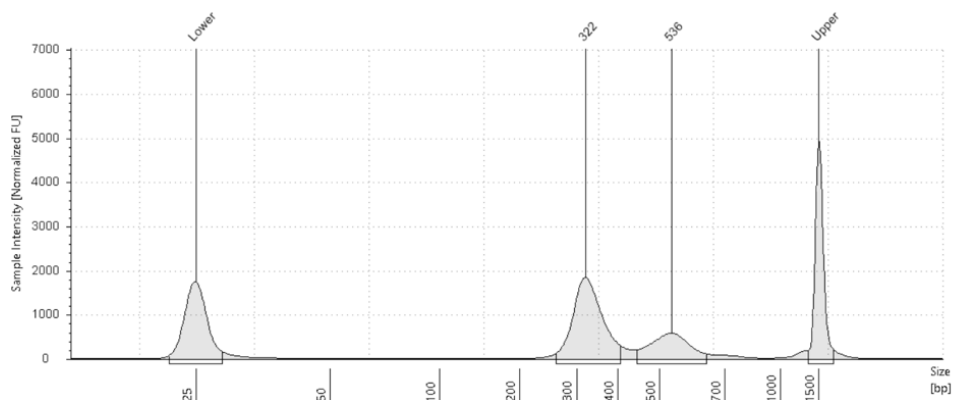


Figure 2. Representative library size distribution generated from 10 ng cfDNA using the **SuperMethyl™ All-in-One Kit** for Illumina®.

[illegible]