Tech Spotlight
Versatile Methyl-seq

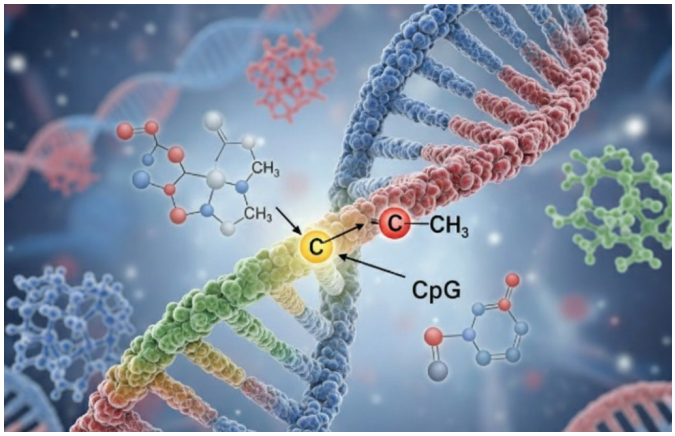
Versatile, robust methylation profiling with the KAPA EvoPrep Kit

Broad compatibility with enzymatic and bisulfite conversion chemistries

DNA methylation is an epigenetic modification that impacts gene expression. Next-generation methylation sequencing (methyl-seq) is a powerful technique for analyzing methylation patterns associated with cancer; particularly for the research of early detection and minimal residual disease (MRD) monitoring.[1]

Methyl-seq workflows require the conversion of unmethylated cytosines to uracil, while (hydroxy)methylated cytosines (5hmC and 5mC) remain unaffected. Chemical conversion is achieved through treatment with sodium bisulfite (**bisulfite sequencing**). Newer, less-biased enzymatic methods (**enzymatic methyl-seq** or **EM-seq**) rely on a combination of methylation-sensitive and methylation-dependent enzymes (e.g., TET enzymes and DNA deaminases) to selectively modify cytosines based on their methylation state. Both approaches require highly efficient library preparation methods to ensure accurate methylation profiling.

[1] *Ju L, et al. Epigenetic insights into minimal residual disease detection in cancer. Genes & Diseases, 2025.*



Here, we demonstrate broad compatibility of the high-performance, automation-friendly KAPA EvoPrep Kit with different conversion chemistries for robust and versatile streamlined methylation sequencing.

Highly efficient library prep and conversion ensure the high coverage depth and uniformity needed for reliable methylation calls

Methyl-seq sample preparation workflows

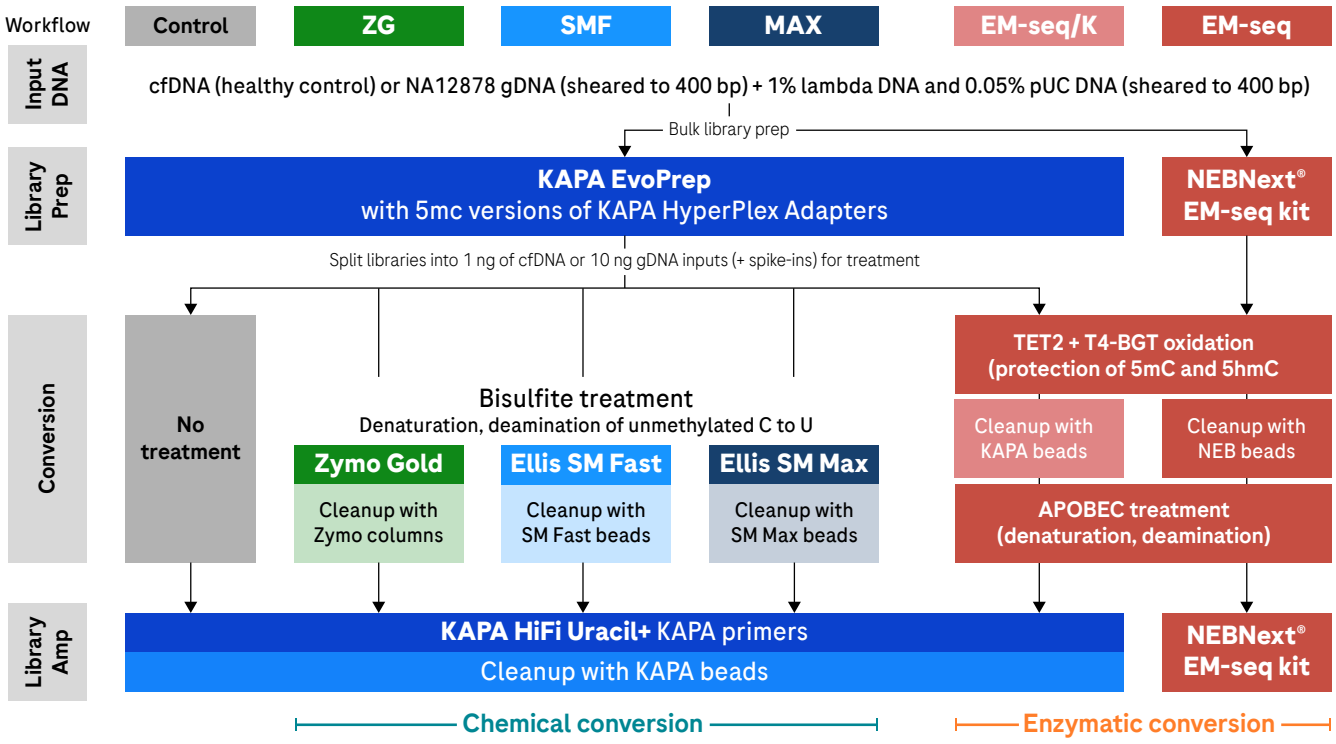


Figure 1. Sample preparation workflows for whole-genome and targeted methyl-seq. A total of six sample preparation workflows were used to generate whole-genome libraries for methyl-seq, including an untreated (unconverted) control. To limit technical variation, bulk libraries were prepared and aliquoted into 1 ng (cfDNA) or 10 ng (gDNA) inputs for conversion with three different chemical and one enzymatic conversion method (n=3 per method). Converted libraries were subsequently combined into multiplex sample pools for enrichment with an internal research panel designed to capture CpG-rich regions, with each pool containing samples generated with all of the workflows (see Figure 3C). A workflow key and additional experimental details are given on the next page.

Data on file at Roche.

Sample preparation workflow key

Workflow	Control	ZG	SMF	MAX	EM-seq/K	EM-seq v2*
Library prep kit	KAPA EvoPrep Kit with custom-synthesized, methylated version of KAPA Universal UMI Adapter (33 µM stock) and 15 min ligation time					NEBNext® Ultra™ II
Conversion chemistry	None	**Chemical (bisulfite sequencing)**			**Enzymatic (EM-seq)**	
Conversion kit	None	EZ DNA Methylation-Gold™ Kit (Zymo Research)	SuperMethyl™ Fast Bisulfite Conversion Kit (Ellis Bio)	SuperMethyl™ Max Bisulfite Conversion Kit Early Access (Ellis Bio)	NEBNext Enzymatic Methyl-seq v2 Kit (New England Biolabs) with KAPA HyperPure Beads for cleanup	NEBNext Enzymatic Methyl-seq v2 Kit (New England Biolabs)
Library amplification	KAPA HiFi Uracil+ Kit and KAPA UDI Primer Mixes (1 – 384)					NEBNext Q5U® Master Mix + NEBNext LV UDI primers Set 1
Library amp cycles	11 cycles for cfDNA 8 cycles for gDNA	14 cycles for cfDNA (1 ng inputs), 11 cycles for gDNA (10 ng inputs)				
Total workflow time	⏱	⏱⏱⏱	⏱	⏱⏱	⏱⏱⏱	⏱⏱⏱
Reason for including	Control	Gold standard for bisulfite conversion, compatible with KAPA EvoPrep Kit	New bisulfite conversion chemistry (launched in 2024)	New bisulfite conversion chemistry (early access)	Established EM-seq workflow with modifications to improve efficiency	Established EM-seq workflow

*EM-seq v2 workflow, performed according to the manufacturer's recommended protocol with reagents provided in the NEBNext Enzymatic Methyl-seq v2 kit.

Key library quality and sequencing metrics

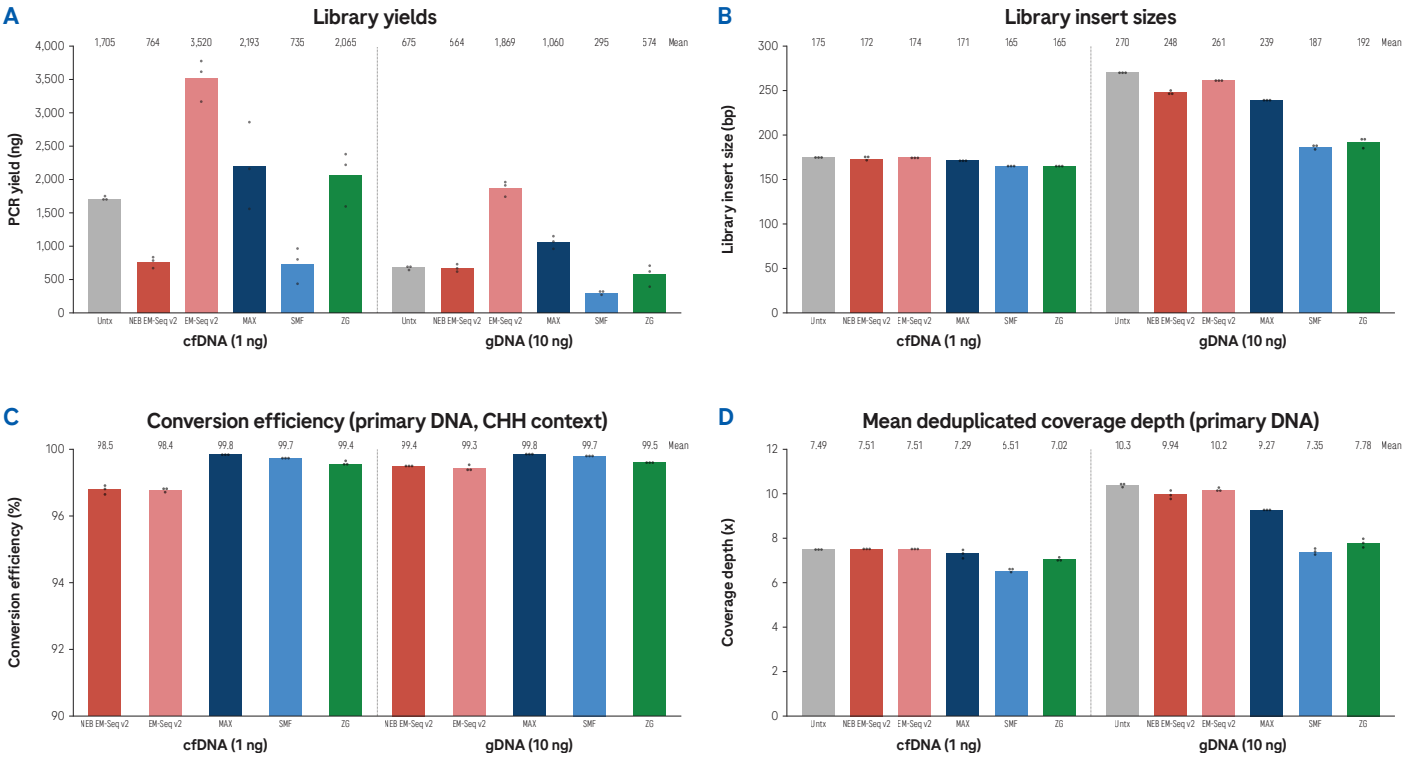


Figure 2. Key library quality and sequencing metrics from whole-genome methylation profiling with the KAPA EvoPrep Kit and different conversion chemistries. **(A)** The modified NEBNext EM-seq v2 workflow ("EM-seq/K," using KAPA purification beads and amplification reagents) resulted in significantly improved library yields over the standard ("EM-Seq v2") protocol. The SuperMethyl Max workflow ("MAX") from Ellis Bio matched or outperformed the gold standard bisulfite conversion kit from Zymo Research ("ZG"). **(B)** All conversion chemistries preserved the insert size of cfDNA libraries. Conversion of gDNA libraries resulted in a reduction of insert size, but not to the extent that impacted downstream sequencing. **(C)** Highly efficient (>98%) conversion of unmethylated primary cfDNA and gDNA minimizes false methylation calls to support reliable methylation profiling. In the CHH sequence context (unmethylated C, followed by an A, T, or C), a conversion rate of approximately 100% is expected. **(D)** Mean deduplicated coverage depths enable accurate methylation calls, even for low-nanogram (≤10 ng) DNA inputs. Coverage depths correlated well with theoretical values expected from the amount of sequencing data used for the analysis (e.g., for libraries prepared from primary cfDNA and gDNA, theoretical coverage was 8.8x). Bulk libraries were prepared from NA12878 reference DNA (Coriell Institute for Medical Research; mechanically sheared to an average fragment length of 400 bp) or cfDNA from a healthy donor. Unmethylated lambda DNA and CpG-methylated pUC19 DNA (also sheared to 400 bp) were spiked into each primary DNA sample, to a final concentration of 1% and 0.05% (m/m), respectively. Libraries were prepared and chemical or enzymatic conversion performed as outlined in the above table. Manufacturer's instructions were followed unless specified otherwise. Library insert sizes were determined with the Agilent® High Sensitivity D1000 DNA ScreenTape assay. Sequencing (2 x 150 bp) was performed on an Illumina® NovaSeq™ instrument (S4 flow cells). Raw FASTQ files were downsampled to an average of 196 million reads per library. Data analysis was performed using an internal research analysis pipeline.

Data on file at Roche.

Versatile and robust methyl-seq performance

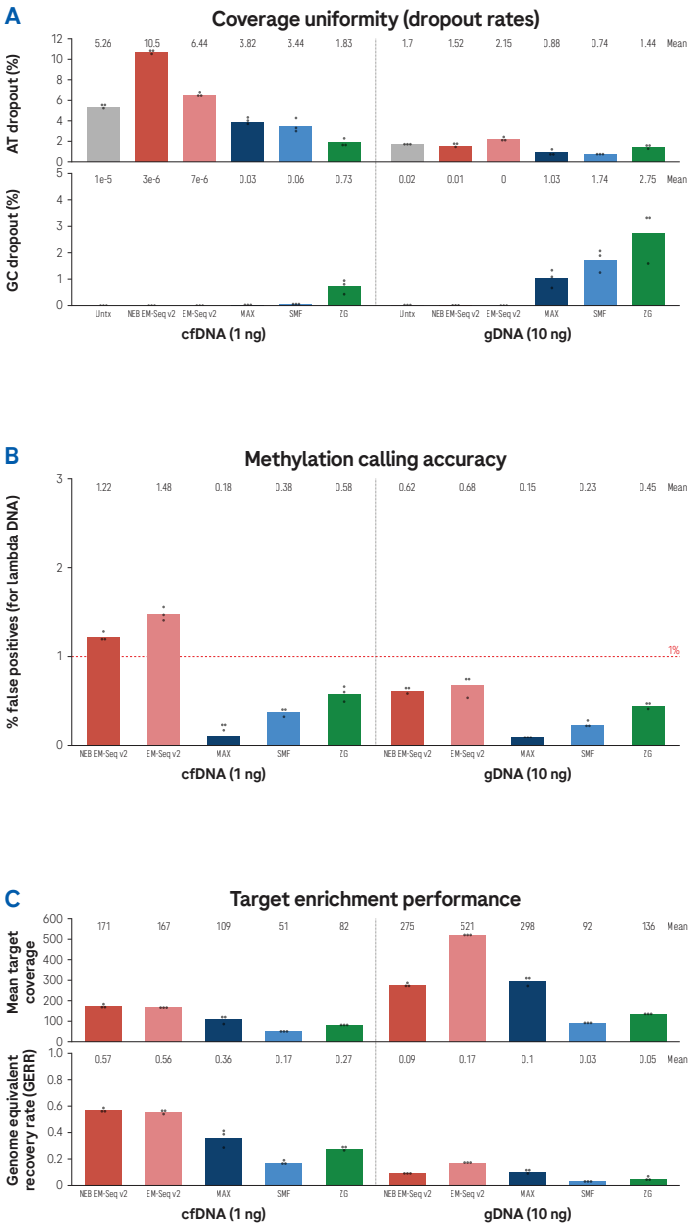


Figure 3. Key performance metrics from whole-genome and targeted methylation profiling with the KAPA EvoPrep Kit and different conversion chemistries. **(A)** Minimal dropout of AT- and particularly GC-rich regions (<3% for all workflows) indicates high coverage uniformity (minimal sequencing bias), even for this challenging application. Enzymatic conversion is known to better conserve GC-rich regions, which is critical for accurate methylation analysis. **(B)** High library yields, efficient conversion, and deep and uniform coverage support accurate methylation calls. False positive rates (unmethylated Cs in CpG islands that were called as Cs, but should have been converted to Ts) were calculated for lamda DNA spiked into primary samples. **(C)** Targeted methyl-seq enables significantly higher coverage depth from the same amount of sequencing data by enriching for CpG islands. Multiplex sample pools (configured as outlined in Figure 1, total of 1.5 µg of converted library DNA per pool) were enriched using an internal research panel designed to capture CpG-rich regions. The KAPA HyperCap Evolved Workflow v5.0 was used with the following minor modifications: (i) a total of 20 µL Human COT DNA, supplemented with other, experimental on-target enhancing oligos (instead of 20 µL Human COT DNA alone), were added to each sample pool in preparation for hybridization, (ii) the amount of Component H in the Hybridization Master Mix was increased to ~40% (17.9 µL in a total volume of 42.6 µL; thus increasing the total hybridization reaction volume inclusive of probes to 63.6 µL). Post-hybridization amplification (16 cycles) was performed with KAPA HiFi HotStart ReadyMix and final libraries were recovered in 25 µL 10 mM Tris-HCl buffer + 0.1% Tween-20. Samples were sequenced to near saturation (approximately 300 million reads/library), indicating that the amount of sequencing may be reduced without compromising sensitivity or accuracy. EM-Seq, which is deemed to be less harsh than bisulfite sequencing, yielded higher genome equivalent recovery rates (GERR). The GERR measures the efficiency of molecule recovery after target enrichment, by quantifying the total number of unique genome equivalents (distinct original DNA molecules) successfully converted into the final sequencing library. A higher GERR is critical, as it signifies a library with greater complexity that can achieve higher maximum non-redundant coverage for reliable results, while a low GERR indicates significant complexity loss due to issues like degradation or PCR bias. This metric is especially important in methylation sequencing because the harsh bisulfite conversion step severely fragments and degrades the DNA, causing a dramatic reduction in the effective number of recovered genome equivalents and thus dictating the usable coverage (watch this video for more information about GERRs).

Summary

The KAPA EvoPrep Kit enables the preparation of **high-quality libraries** from a variety of sample types for a broad range of applications. **Demonstrated compatibility** with **enzymatic** and **bisulfite conversion chemistries** make it the ideal library preparation solution for methyl-seq workflows.

In both bisulfite and EM sequencing workflows, **consistently high (>98%) conversion efficiency** of KAPA EvoPrep libraries can be achieved, even from **low inputs (≤10 ng)** and **challenging samples** such as **cfDNA**. This is essential to minimize false calls from unconverted, non-methylated cytosines and ensure **accurate methylation profiling**.

Despite a typical reduction in library fragment length during the conversion of libraries for methyl-seq, all of the workflows demonstrated here **preserve library fragment lengths sufficiently** for downstream sequencing.

Methyl-seq sample preparation workflows employing the KAPA EvoPrep Kit produces highly **complex libraries with minimal GC bias**, which translates to **excellent sequence coverage uniformity**.

High coverage depth enables reliable methylation analysis of libraries prepared from low inputs of gDNA and cfDNA, using both whole-genome and targeted sequencing approaches.

Learn more...

about the **KAPA EvoPrep Kit** and how it can enable you to prepare the highest quality libraries for methylation analysis.

Click **here** or scan the code below:

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