Tech Spotlight

Robust Bisulfite Sequencing

Bisulfite sequencing with KAPA EvoPrep Kits and Zymo EZ DNA Methylation-Gold™

Compatibility for robust methylation profiling

DNA methylation profiling, particularly the detection of the powerful epigenetic marker, 5-methylcytosine (5mC), is becoming increasingly important in the research for early cancer detection and minimal residual disease (MRD) monitoring.

Bisulfite sequencing, which involves the conversion of unmethylated cytosines to uracils, requires library preparation methods that maintain DNA integrity and yield high-quality, conversion-ready libraries. Here, we show results for a reliable, streamlined workflow that combines the highly efficient, automation-friendly KAPA EvoPrep Kit for library prep and the EZ DNA Methylation-Gold™ Kit from Zymo Research.

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

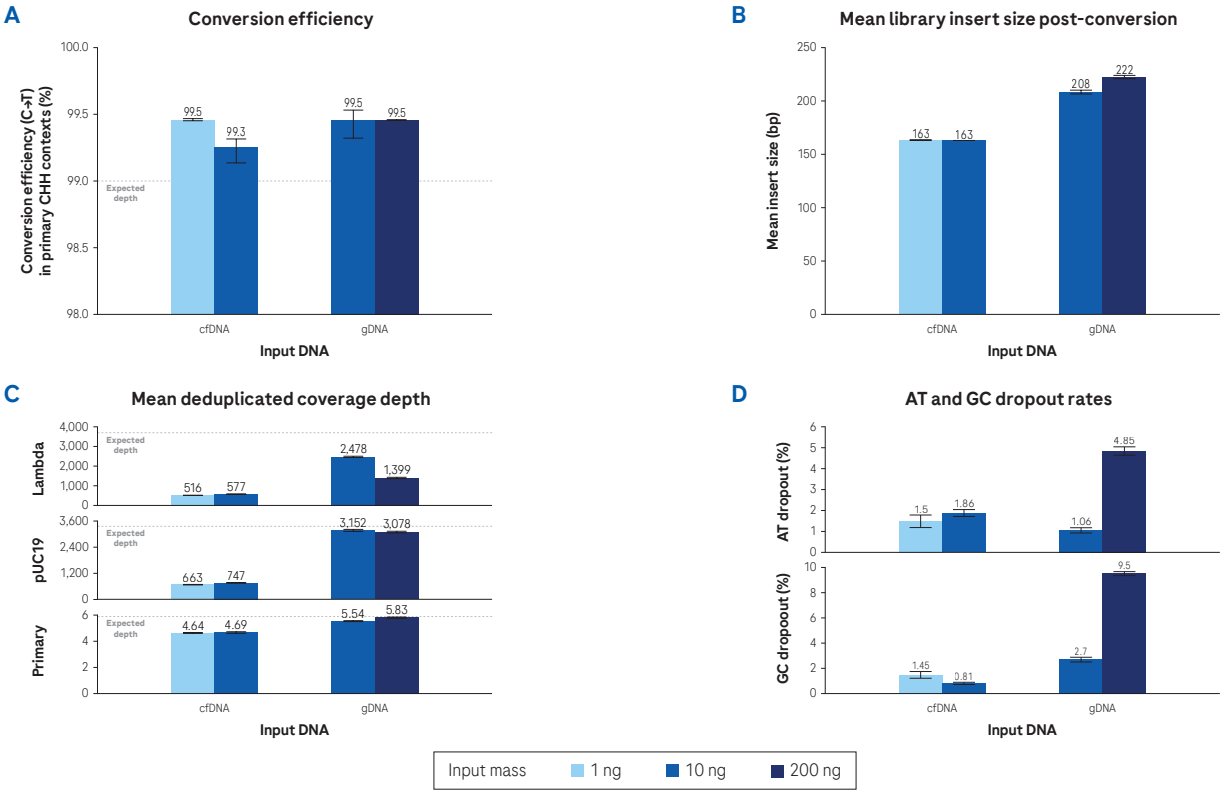

Figure 1. The KAPA EvoPrep Kit and EZ DNA Methylation-Gold™ Kit from Zymo Research ("Zymo Gold kit") support robust bisulfite sequencing. (A) Highly efficient (>99%) conversion of unmethylated primary cfDNA and gDNA minimizes false methylation calls to support reliable methylation profiling. In the CHH sequence context (unmethylated C, followed by an A, T, or C), a conversion rate of approximately 99% is expected. (False positive rates, calculated from an unmethylated lambda DNA spike-in, were <0.5% and <1.6%, respectively, for the lowest gDNA and cfDNA inputs; data not shown). **(B)** Mean library insert sizes after bisulfite conversion are sufficient for downstream sequencing. **(C)** Mean deduplicated coverage depths enable accurate methylation calls, even for low-nanogram (≤10 ng) 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 100 ng gDNA, theoretical values were 3,711x for the lambda DNA spike-in, 3,350x for pUC19 spike-in, and 5.9x for the primary DNA). **(D)** Minimal dropout of AT- and GC-rich regions (<3% across all conditions) indicates high coverage uniformity (minimal sequencing bias), even for this challenging application. Preservation of GC-rich regions is particularly important for accurate methylation analysis. **Methods overview:** Libraries for whole-genome bisulfite sequencing (WGBS) were prepared in triplicate from 10 or 200 ng of NA12878 reference DNA (Coriell Institute for Medical Research; mechanically sheared to an average fragment length of 400 bp) and 1 or 10 ng of cfDNA from a healthy donor. Unmethylated lambda DNA and CpG-methylated pUC19 DNA (also sheared to 400 bp) were spiked into each sample to a final concentration of 1% and 0.05% (m/m), respectively, as controls for conversion efficiency. Libraries were prepared with the KAPA EvoPrep Kit, using a custom-synthesized mC version of the KAPA Universal UMI Adapter (33 µM stock), standard KAPA UDI Primer Mixes, 15 min of ligation time, and the KAPA HiFi HotStart Uracil+ ReadyMix for library amplification (7 – 14 cycles, depending on input). Bisulfite conversion was performed with the Zymo Gold kit as per the manufacturer's instructions. Sequencing (2 x 150 bp) was performed on an Illumina® NovaSeq™ instrument (S4 flow cells). Data were downsampled to an average of 196 million total reads per library. Data analysis was performed using an internal RUO analysis pipeline.

Data on file at Roche.

KAPA EvoPrep + EZ DNA Methylation-Gold™ Library Prep workflow

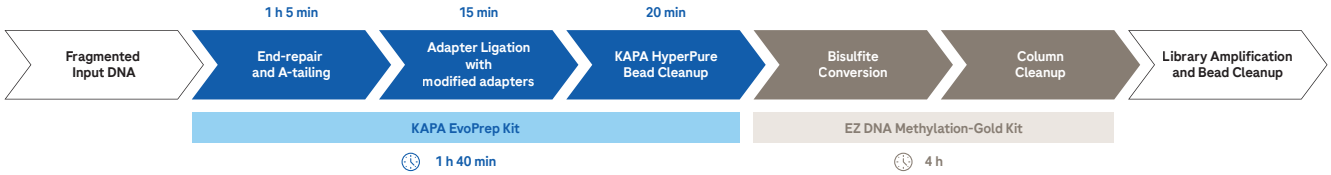

Figure 2. Streamlined WGBS library preparation workflow with the KAPA EvoPrep and Zymo Research EZ DNA Methylation-Gold Kits. Two modifications were made to the KAPA EvoPrep library preparation protocol, to optimize library yields prior to bisulfite conversion, particularly for low-input and challenging samples (standard protocol could also be followed): (i) an extended ligation time (15 min instead of the standard 5 min), and (ii) a higher adapter stock concentration (33 µM instead of 15 µM). **Notes on methods:** adapters **with methylated cytosines** and library amplification with the uracil-tolerant **KAPA HiFi HotStart Uracil+ DNA Polymerase** are required for this application. The Zymo Research EZ DNA Methylation-Gold Kits streamlines the conversion of unmethylated cytosines (C) to uracils (U) with (i) a coupled heat denaturation/conversion reaction, and (ii) column-based DNA purification and desulphonation. During library amplification and sequencing, uracils are read as thymines (T). Since methylated cytosines (mC) are not modified, researchers are able to distinguish between methylated and unmethylated cytosines by comparing treated and untreated DNA sequences. The time for each step reflects total turnaround time (hands-on plus incubation times), confirming that sequencing-ready WGBS libraries can be prepared with the KAPA EvoPrep-Zymo Gold workflow in a standard workday.

Summary

Bisulfite treatment of KAPA EvoPrep libraries with the EZ DNA Methylation-Gold™ Kit from Zymo Research offers **consistently high (>99%) conversion efficiency**, 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 resulting from the harshness of bisulfite treatment, the KAPA EvoPrep-Zymo Gold workflow **preserves library fragment lengths sufficiently** for downstream sequencing.

ATCGGCA
TTTGGCC
CGCATCG
GACTACG

The KAPA EvoPrep-Zymo Gold workflow produces highly **complex libraries with minimal GC bias**, which translates to **excellent sequence coverage uniformity**.

High deduplicated sequencing coverage depth enables reliable methylation analysis for WGBS libraries prepared from low inputs of gDNA and cfDNA, with only 200 million reads per library.

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:

Data on file at Roche.
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