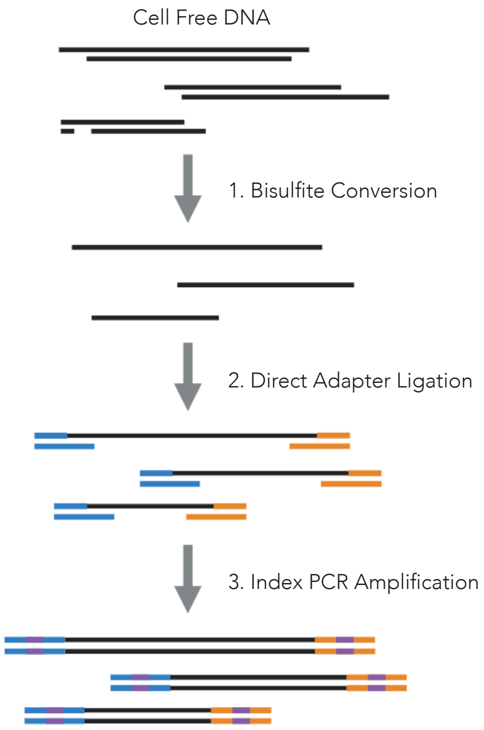
ZYMO RESEARCH
The Beauty of Science is to Make Things Simple®

High Quality Libraries from Precious cfDNA Samples

Zymo-Seq™ Cell Free DNA WGBS Library Kit

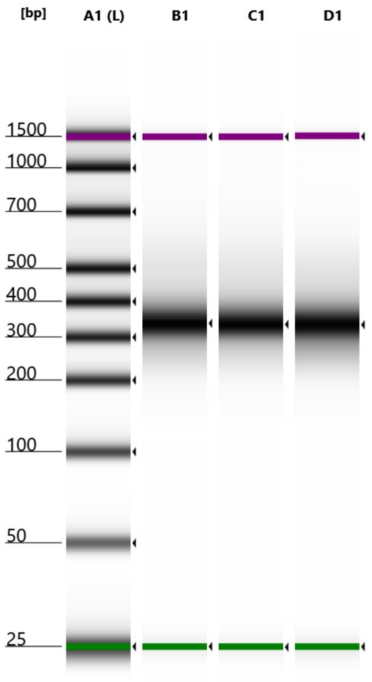
- **Optimized for Small Fragment Input:** Ideal for small and damaged DNA fragments such as cell-free DNA (cfDNA).
- **Accurate Methylation Calling:** Direct ligation-based protocol allows for accurate reads and methylation calling of native termini for each DNA fragment.
- **Simple, Streamlined Workflow:** Prepare robust methyl-seq libraries in as little as 3 steps.

Optimized and Simple Workflow



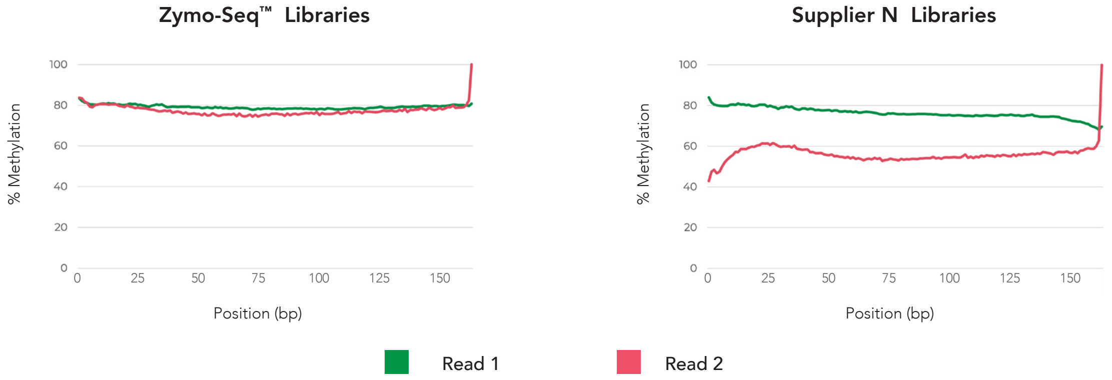
Overview of the Zymo-Seq™ Cell Free DNA WGBS Library Kit protocol. The cfDNA is first bisulfite converted using optimized conditions for fragmented input. Next, the innovative adapters capture and directly ligate onto any size DNA fragment, thus accurately preserving the methylation status of each terminus. Finally, the adapter ligated cfDNA is indexed and amplified via PCR, and the libraries are ready for sequencing on any Illumina instrument.

High Quality cfDNA Libraries



Zymo-Seq™ Cell Free DNA WGBS libraries prepared from multiple cfDNA samples. Agilent 4200 TapeStation HS D1000 gel of libraries prepared using cfDNA extracted from plasma of healthy and cancerous donors. A1 is the molecular weight marker. B1 was prepared from a healthy 59-year-old donor. C1 was prepared from a lung cancer NSCLC stage IV 66-year-old donor. D1 was prepared from an adenocarcinoma stage IV 69-year-old donor. All libraries were generated using 5 ng input cfDNA and amplified at 9 index PCR cycles.

Accurate Methylation Calling Across the Entire Read



Zymo-Seq™ Cell Free DNA libraries minimize library preparation bias commonly found in conventional methods. Unbiased libraries will have consistent methylation levels across the entire read length. Other commercial protocols that include an end-repair step incorporate artificial nucleotides to blunt damaged DNA termini, resulting in significant methylation bias on the 3' end of the DNA fragments. The Zymo-Seq™ Cell Free DNA WGBS Library Kit directly ligates the adapters, eliminating the need for end-repair and thus preserving the integrity of native methylation present on the fragment termini. The Zymo-Seq™ Cell Free DNA library (left) shows consistent CpG methylation across both Read 1 and Read 2 whereas the Supplier N library (right) shows significant bias. The M-Bias plots shown above were generated by plotting the average CpG methylation levels across each position of the mapped read.

Zymo-Seq™ Cell Free DNA WGBS Kit Specifications

Feature	Zymo-Seq™ Cell Free DNA
Sample Type	Cell free DNA, fragmented DNA, gDNA (adaptable)
Equipment	Thermal cycler(s), microcentrifuge, magnetic stand
Reagents	All inclusive
Max UDI	96
Input Amount	> 5ng
Total Assay Time	~6 hours
Hands-On Time	~2 hours
FFPE Compatible	Yes
Compatible Sequencing Platforms	All Illumina instruments. Recommended: HiSeq, NextSeq, NovaSeq

Superior Performance

Metric	Zymo-Seq™ Cell Free DNA
% BS Conversion	99.6%
% Aligned	81.5%
Median Insert Size	158 bp
% CpG Coverage > 5x	81.9%
% Promoter Coverage > 50x	92.1%
% CpG Island Coverage > 50x	92.7%

Library was prepared from 5 ng input cfDNA and sequenced via NovaSeq 6000 with approximately 400M PE sequencing reads. Reads were aligned to hg38 using Bismark and methylation calling was performed with MethylDackel.

Product	Cat. No.	Size
Zymo-Seq™ Cell Free DNA WGBS Library Kit	D5462 D5463	24 preps 96 preps