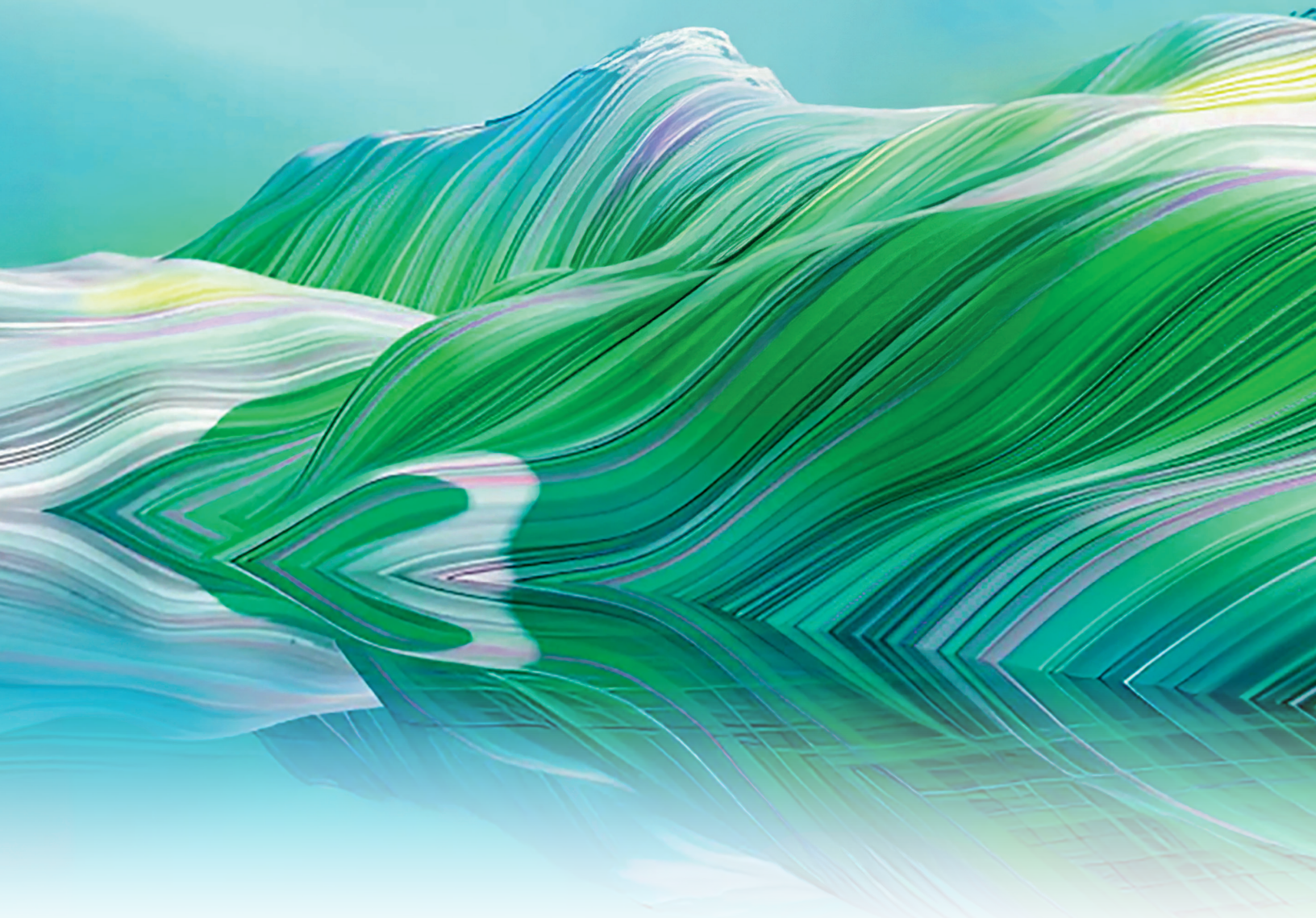
ZYMO RESEARCH

The Beauty of Science is to Make Things Simple®

THE Epigenetics COMPANY™

BEYOND EPIGENETIC HORIZONS

Explore Cutting-Edge Tools and Technologies for Navigating the Epigenetic Landscape



Epigenetics

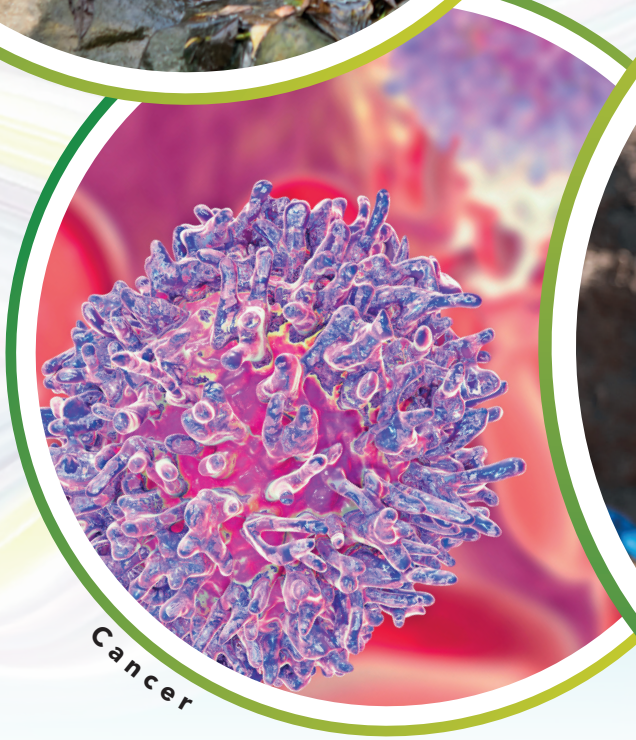
Plant

Aging

Microbiomics

Environmental

Epigenetics is revolutionizing our understanding of biology, offering profound insights into how genes are regulated without altering DNA sequences. This dynamic field spans countless research areas, from unraveling the mysteries of cancer and neurodegenerative diseases to optimizing regenerative medicine and agricultural resilience. Epigenetic modifications shape development, influence aging, and even mediate environmental responses, bridging the gap between nature and nurture. The potential of epigenetics is limitless, redefining science and opening new frontiers in health, disease prevention, and beyond.



Cancer

Archaeogenetics

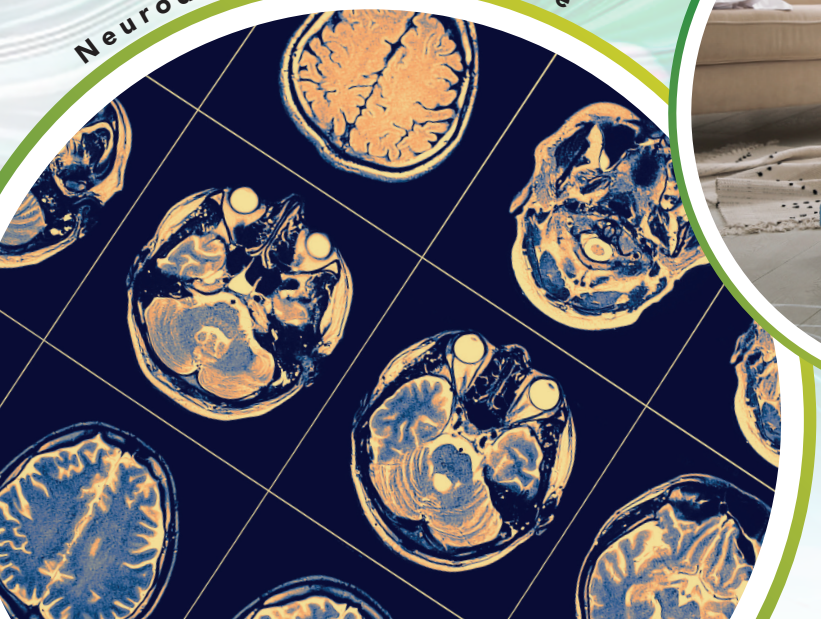
Animal Biology

Exercise Science

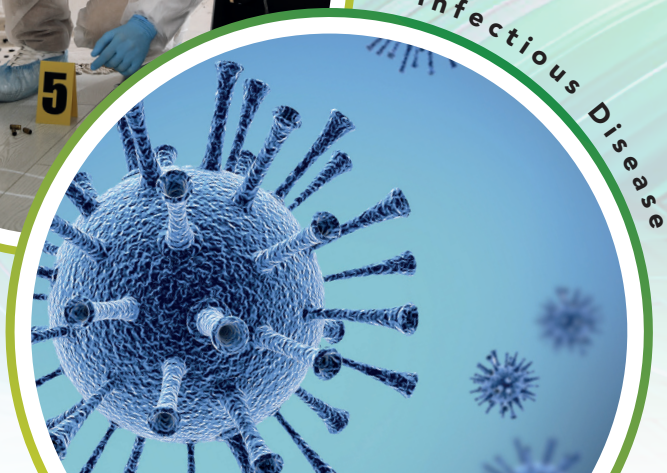
Space Biology

The study of epigenetics continues to unlock new dimensions of biological complexity. As we deepen our understanding of epigenetic mechanisms, we move closer to groundbreaking discoveries in medicine, wildlife conservation, and beyond. With ongoing research using cutting-edge technology, epigenetics holds the key to addressing some of the most pressing challenges of our time, paving the way for a healthier, more resilient future.

Forensics



Neurodegenerative Disease



Infectious Disease

Epigenetics

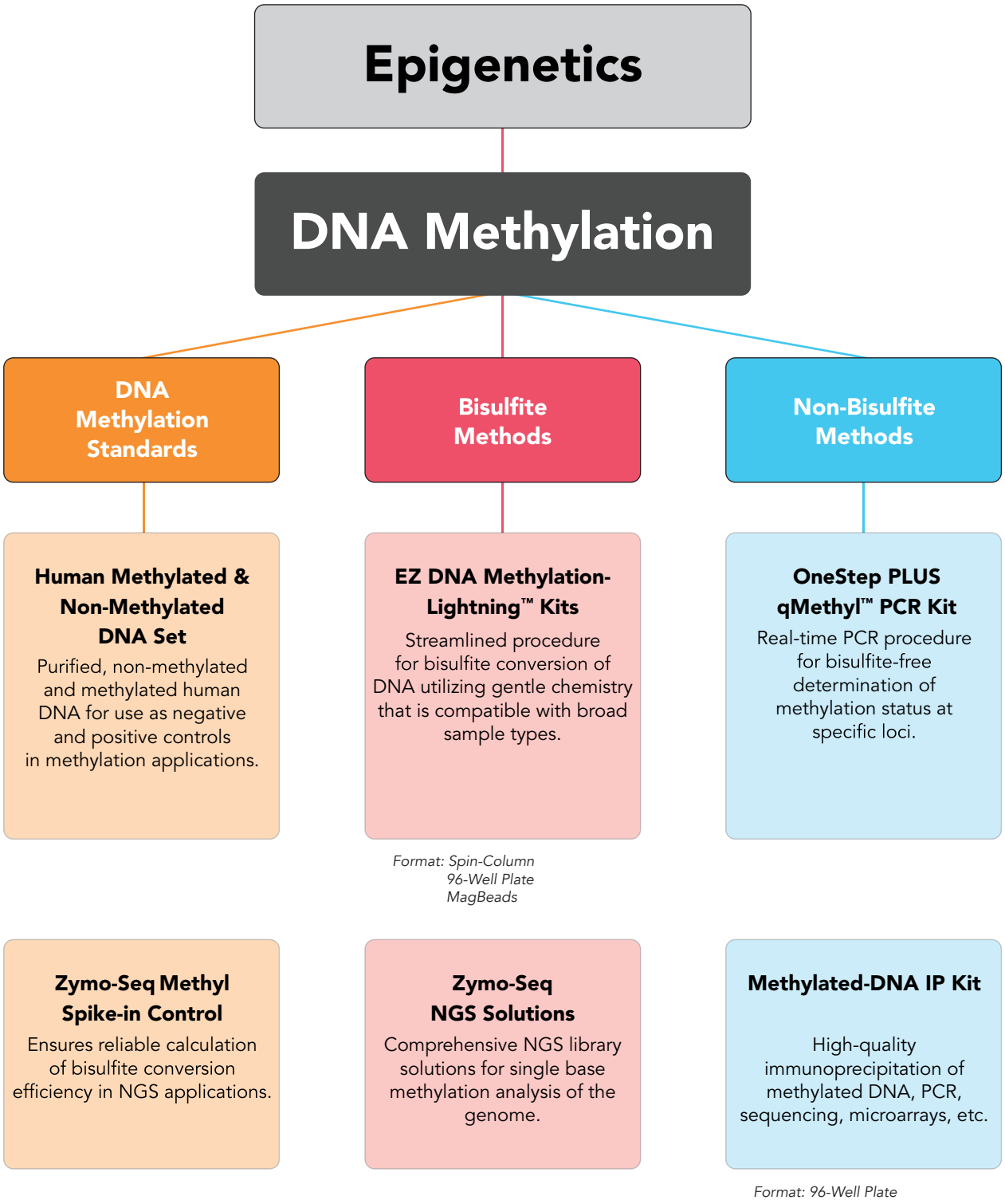
Epigenetics, from the Greek “epi” meaning “on top of” or “over,” delves into regulatory mechanisms beyond the primary sequence of DNA. Established over seventy years ago, it has transformed our understanding of development, differentiation, and disease mechanisms.

As “The Epigenetics Company”, Zymo Research is the leader in products and services for epigenetics-focused research. Our DNA methylation technologies, including EZ DNA Methylation™ kits and controls, are the gold standard for clinical studies. Zymo Research offers next-generation sequencing (NGS) solutions that set new standards for both speed and accuracy in methylation and chromatin profiling.

We offer advanced chemistries, automated and certified workflows, and expert data support for rapid and reliable analyses. Zymo Research provides epigenetic programs for exploring genetic/epigenetic regulation in embryology, aging, cancer, and beyond.

THE Epigenetics COMPANY™

Quick Product Guide



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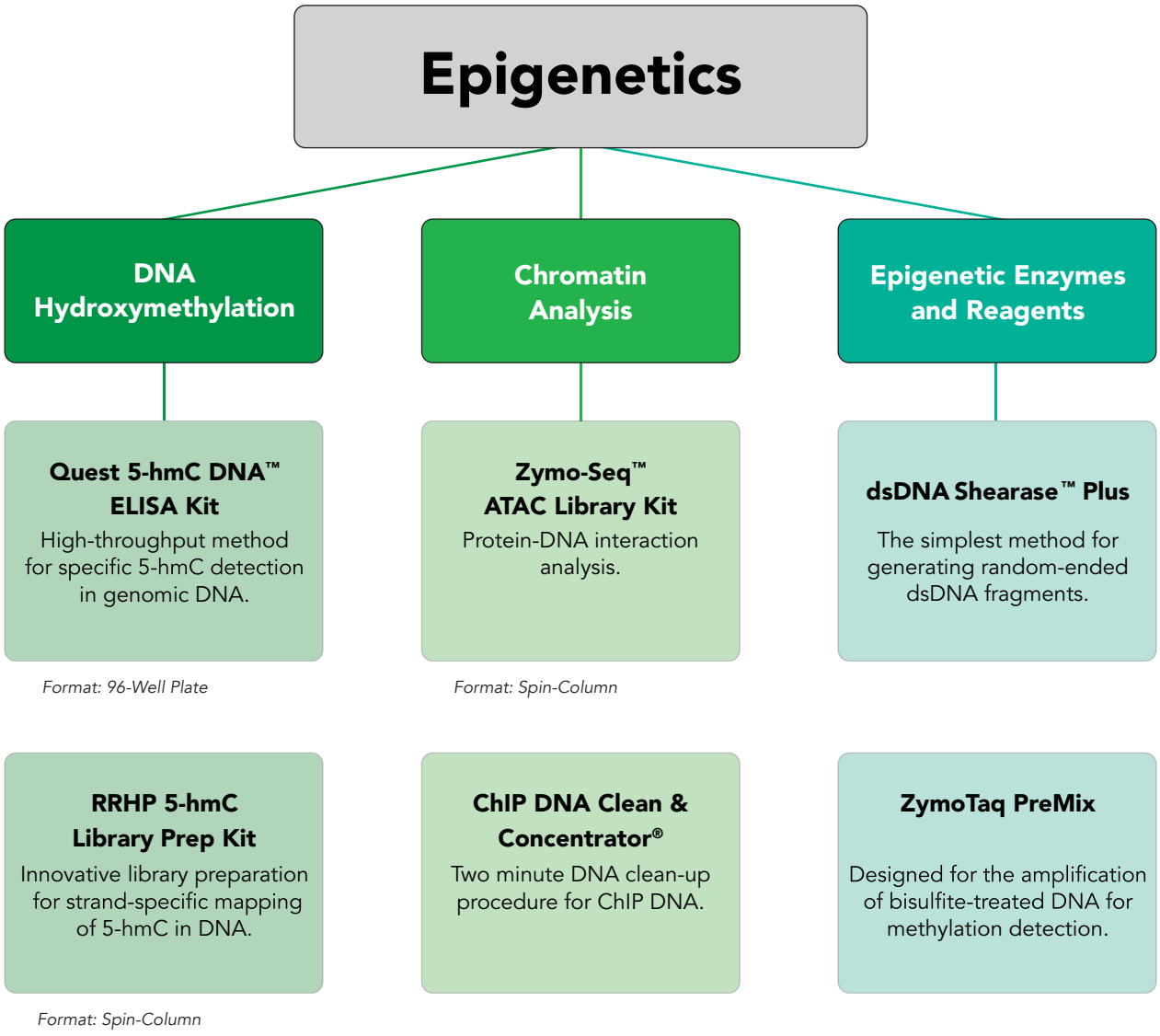
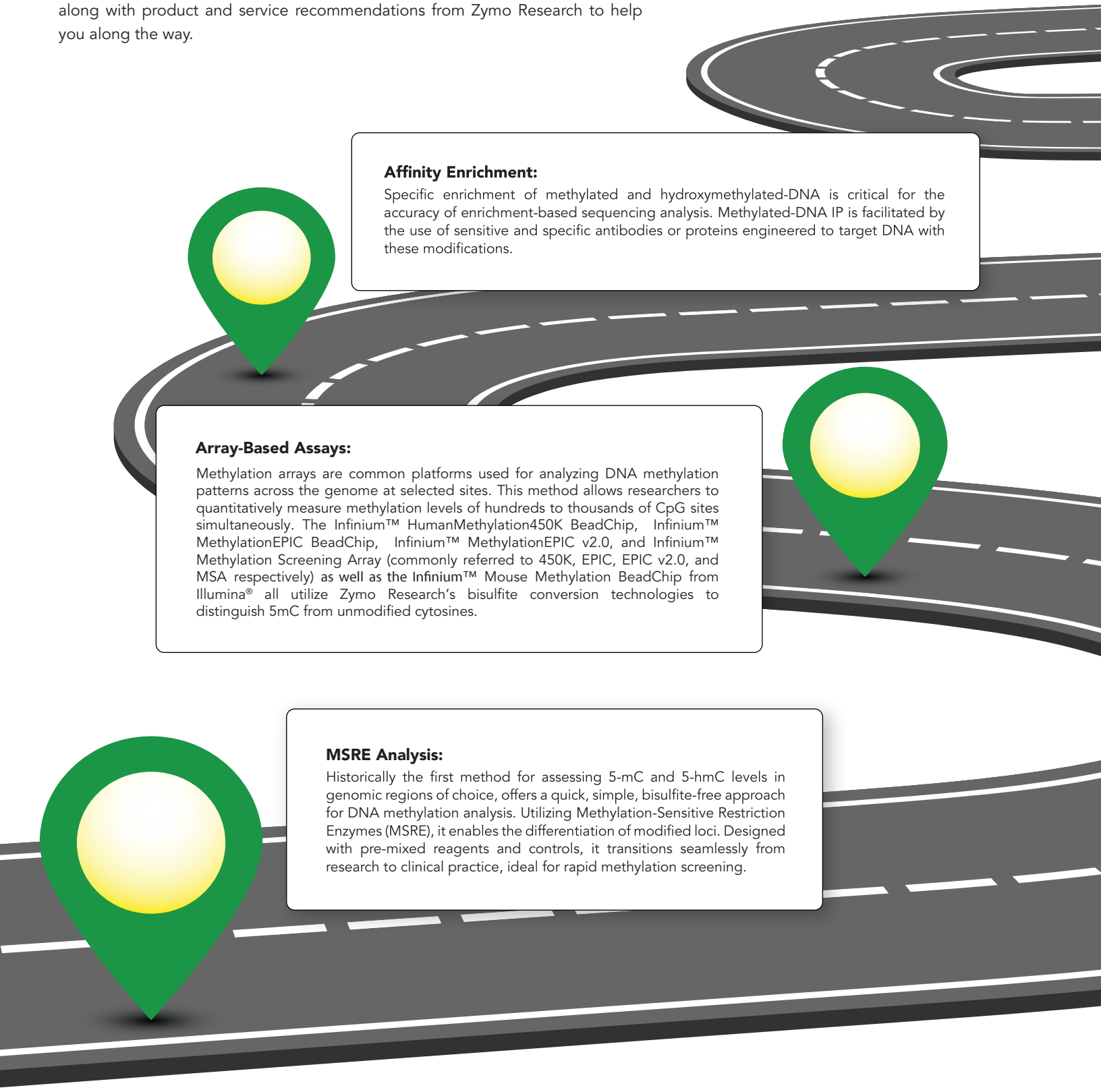
Quick Product Guide



A Roadmap for Navigating the Epigenetic Landscape

Epigenetic analysis doesn't have to be complicated. The scientists at Zymo Research have developed this navigation tool to assist both new and experienced researchers in tackling epigenetic analysis with ease. Below you will find an overview of the most commonly used techniques for studying DNA methylation, along with product and service recommendations from Zymo Research to help you along the way.



Affinity Enrichment:

Specific enrichment of methylated and hydroxymethylated-DNA is critical for the accuracy of enrichment-based sequencing analysis. Methylated-DNA IP is facilitated by the use of sensitive and specific antibodies or proteins engineered to target DNA with these modifications.

Array-Based Assays:

Methylation arrays are common platforms used for analyzing DNA methylation patterns across the genome at selected sites. This method allows researchers to quantitatively measure methylation levels of hundreds to thousands of CpG sites simultaneously. The Infinium™ HumanMethylation450K BeadChip, Infinium™ MethylationEPIC BeadChip, Infinium™ MethylationEPIC v2.0, and Infinium™ Methylation Screening Array (commonly referred to 450K, EPIC, EPIC v2.0, and MSA respectively) as well as the Infinium™ Mouse Methylation BeadChip from Illumina® all utilize Zymo Research's bisulfite conversion technologies to distinguish 5mC from unmodified cytosines.

MSRE Analysis:

Historically the first method for assessing 5-mC and 5-hmC levels in genomic regions of choice, offers a quick, simple, bisulfite-free approach for DNA methylation analysis. Utilizing Methylation-Sensitive Restriction Enzymes (MSRE), it enables the differentiation of modified loci. Designed with pre-mixed reagents and controls, it transitions seamlessly from research to clinical practice, ideal for rapid methylation screening.

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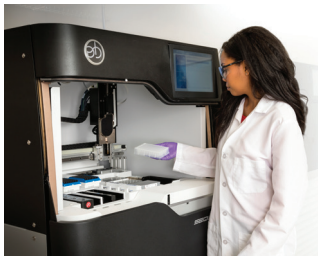
Third-Generation Sequencing for 5-mC Detection:

Third-generation sequencing technologies analyze methylation patterns over extensive regions of the genome directly and without bisulfite conversion. This method provides high-resolution insights into the epigenetic modifications across large genomic landscapes, allowing for the detection of methylation patterns in full-length gene sequences, repetitive elements, and complex genomic structures.



Bisulfite Treatment:

Bisulfite treatment is considered the "gold standard" for the analysis of DNA methylation with supreme efficiency, reproducibility and simplicity. Bisulfite treatment converts unmodified cytosine to uracil while methylated cytosines are protected from this conversion. Downstream analyses include methylation-specific PCR (MSP), bisulfite-specific PCR (BSP), sequencing, hybridization, pyrosequencing and NGS.

Sequencing-Based Assays:

NGS-based methods have emerged as the primary and most comprehensive means of DNA methylation analysis recently. NGS-based methods produce results with high coverages across an entire genome and are compatible with most species. Bisulfite sequencing is thus gaining tremendous popularity among researchers for DNA methylation profiling, indicated by the increasing number of related publications over the years. DNA samples must be both bisulfite converted and prepared into libraries to allow for the differentiation of methylated from unmethylated cytosines present. During sequencing, the methylated cytosines that were protected during the bisulfite treatment will be recognized as "C" in the NGS sequencing data. Unmethylated cytosines that were converted will be recognized as "T" in the data. Using NGS, DNA methylation can be investigated at single-nucleotide resolution for further data analysis.

Thus far, researchers have implemented a myriad of bisulfite sequencing methods to uncover DNA methylation information for diverse biological questions. The most extensively used NGS methods include reduced representation bisulfite sequencing (RRBS), whole-genome bisulfite sequencing (WGBS), and targeted bisulfite sequencing. RRBS achieves DNA methylation profiling at single-nucleotide resolution in CpG-rich regions of the genome, making it ideal for pilot studies. WGBS interrogates the entirety of the genome which allows for a comprehensive analysis of DNA methylation throughout. Targeted bisulfite sequencing involves custom profiling of specific regions that are of interest for the project. Zymo Research's portfolio includes both kits and services for each of these sequencing types.

Start your epigenetics journey today!
Scan this QR code to contact our services team or talk to a scientist.

Zymo-Seq™ Trio WGBS Library Kit

Highlights:

- **Co-Detection of Genetic and Epigenetic Information:** Seamlessly analyze both genomic data and DNA methylation in a single experiment, with the validated open-source bioinformatics tools and comprehensive step-by-step guides.
- **True-End Fragment Analysis:** Designed for optimal performance with small or degraded DNA fragments, including cell-free DNA (cfDNA) and FFPE-derived DNA, to capture the fragment's true end.
- **Accurate Methylation Detection:** Achieve precise methylation calling with a direct ligation-based protocol that preserves native termini, ensuring accuracy in each DNA fragment analysis.

Description:

The Zymo-Seq Trio WGBS Library Kit offers an optimized and reliable workflow for whole genome bisulfite sequencing (WGBS) library preparation from cell-free DNA (cfDNA), FFPE DNA, and gDNA. This all-inclusive kit features a straightforward procedure capable of preparing high-quality methyl-seq libraries from as little as 5 ng of cfDNA or fragmented DNA. The process is completed in three basic steps: bisulfite conversion, direct adapter ligation, and index PCR amplification.

The initial bisulfite treatment is gentle on fragmented DNA, such as cfDNA, yet effective in converting any unmodified cytosines into uracil. Next, the innovative splinted adapters capture and directly ligate the Illumina-compatible adapters onto any size DNA fragment, allowing for sequencing of nicked, damaged, and short DNA fragments that may have otherwise been discarded with traditional library prep methods. The direct ligation of the adapters also eliminates the need for second-strand synthesis, end repair, and dA tailing steps, thus reducing bias by preserving the integrity of any native methylation present on the fragment termini. Finally, validated open-source bioinformatic tools and a step-by-step guide are available for customers to co-detect genetic information, enhancing downstream analysis power.

Optimized and Simple Workflow

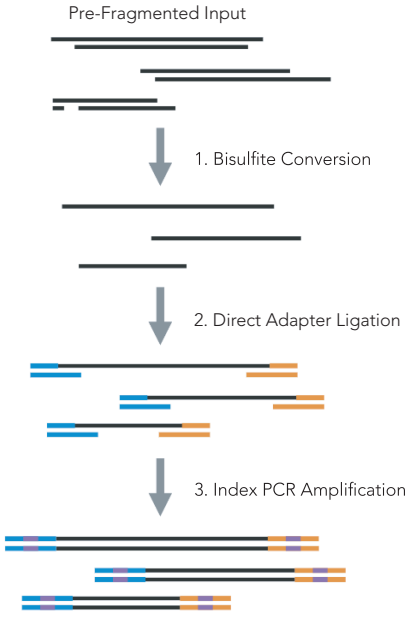


Figure 1: Overview of the Zymo-Seq™ Trio WGBS Library Kit protocol. The simple three-step protocol allows users to effortlessly prepare WGBS libraries from small, damaged DNA with no compromise on quality.

Accurate Methylation Calling Across the Entire Road

Zymo-Seq™ Trio Library Kits 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™ Trio 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™ Trio WGBS Library Kit shows consistent CpG methylation across both Read 1 and Read 2, whereas Supplier N 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.

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

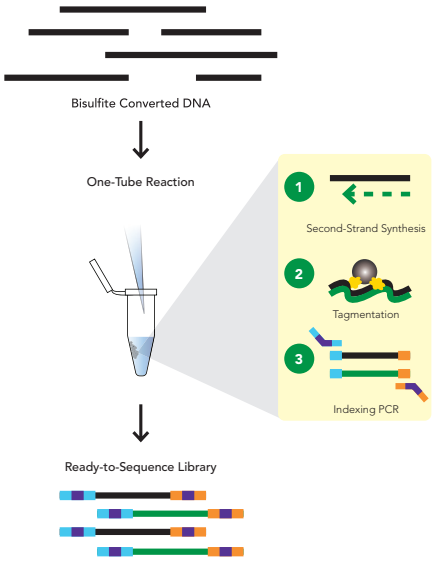
Zymo-Seq™ WGBS Library Kit

Highlights:

- **Bisulfite Library Preparation in One Tube**
- **Streamlined Workflow:** From genomic DNA to ready-to-sequence library in 4 hours.
- **Consistent Genome Coverage:** Unbiased, single-base methylation profiling of cytosines throughout the entire genome.

Description:

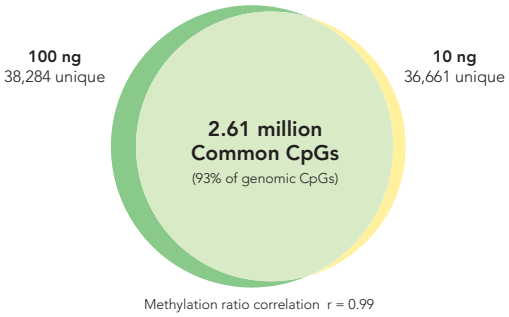
Zymo-Seq WGBS Library Kit is the only kit available for whole genome bisulfite (WGBS) library preparation in a single tube. Zymo-Seq WGBS Library Kit incorporates tagmentation technology to eliminate the tedious fragmentation, enzymatic, and clean-up steps required by conventional, ligation-based library preparation methods. This streamlined workflow minimizes hands-on time, making it an ideal choice for higher throughput applications.



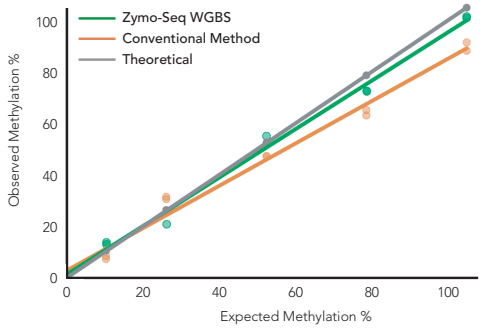
Zymo-Seq WGBS Library Kit Workflow

Enzymatic reactions are consolidated in a single tube to minimize hands-on time. Intact genomic DNA is first bisulfite converted, which then undergoes second strand synthesis (1). Tagmentation is added directly to the reaction to tag adapters onto the double-stranded DNA (2). Indexing primers and PCR mixture are added to amplify the WGBS library (3). Purified libraries are ready for sequencing on Illumina instruments.

Reproducible Coverage and Methylation Detection



Coverage is preserved using as little as 10 ng DNA input. Over 93% of genomic CpG sites overlapped (10X depth cutoff) between Zymo-Seq libraries prepared using 100 ng and 10 ng of Arabidopsis genomic DNA. The two libraries had less than a 10% methylation difference in over 90% of shared CpG sites, demonstrating consistency between different inputs.



Unbiased methylation callings are produced using Zymo-Seq WGBS Library Kit. Spike-ins with known methylation ratios (0%, 10%, 25%, 50%, 75%, and 100%) were added to 100 ng of genomic DNA prior to processing with the Zymo-Seq WGBS Library Kit or using the conventional methodology. The observed methylation % for Zymo-Seq libraries closely matched theoretical methylation levels (R2 = 0.997).

Product	Cat. No.	Size
Zymo-Seq WGBS Library Kit	D5465	24 preps

Zymo-Seq™ RRBS Library Kit

Highlights:

- **Simple Workflow:** Prepare Reduced Representation Bisulfite Sequencing (RRBS) libraries in as little as 2 hours of hands-on time.
- **Low Input:** The only RRBS kit that produces NGS libraries from ≥ 10 ng of genomic DNA.
- **Accurate and Reproducible:** Unbiased methylation calling and reproducible CpG coverage.

Zymo-Seq™ RRBS Library Kit Has A Simple Workflow

Input genomic DNA

↓

5 minutes — MspI digestion

↓

20 minutes — Adapter ligation and gap filling

↓

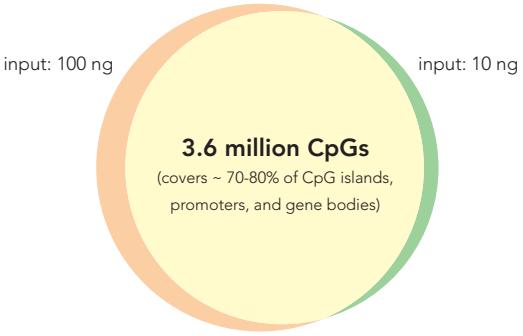
40 minutes — Bisulfite conversion

↓

20 minutes — Index primer amplification

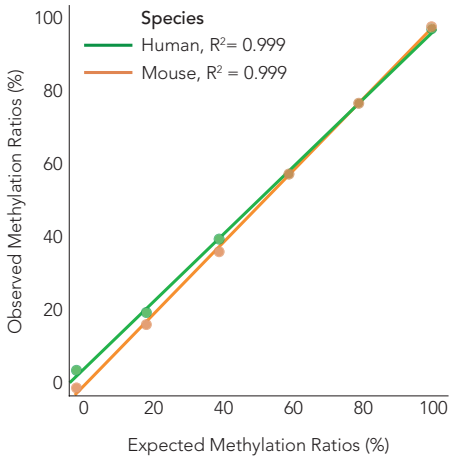
↓

Sequencing on any Illumina platform



*Methylation Ratio Correlation Pearson's r=0.97

Zymo-Seq™ RRBS Library Kit Measures Accurate Methylation Ratios



Libraries were prepared using the Zymo-Seq™ RRBS Library Kit from 100 ng of human or mouse gDNA with known methylation ratios (0%, 20%, 40%, 60%, 80%,100%). The observed methylation ratios closely matched the expected ratios, demonstrating unbiased results acrossall samples.

Zymo-Seq™ RRBS Library Kit Delivers Reproducible Results Regardless of Input Amount

Over 3.6 million CpG sites (≥ 5X read coverage) were sequenced from libraries generated using the Zymo-Seq™ RRBS Library Kit, even when starting with only 10 ng of human genomic DNA. These sites cover a majority of functional regions in the human genome including CpG islands, promoters, and gene bodies.

Product	Cat. No.	Size
Zymo-Seq™ RBBS Library Kit	D5460 D5461	24 preps 48 preps

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Pico Methyl-Seq™ Library Prep Kit

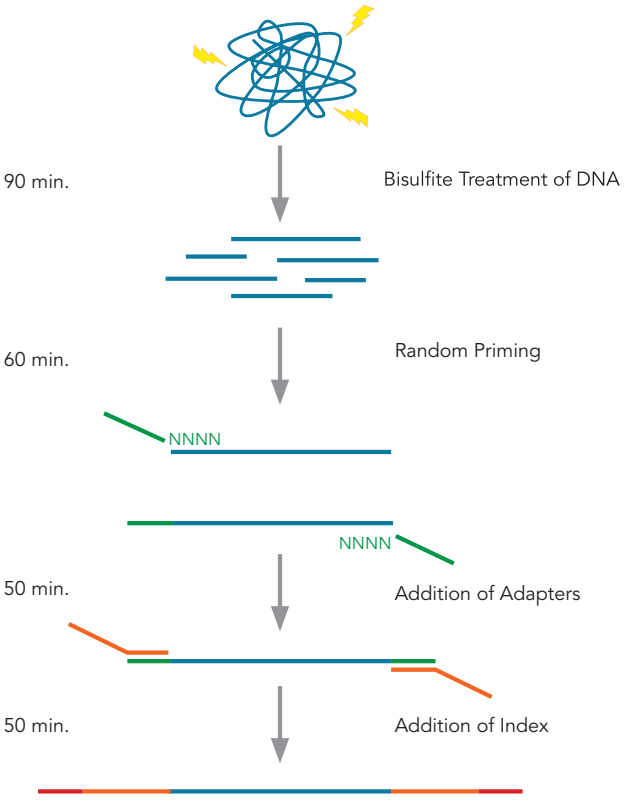
Highlights:

- **All-inclusive:** Complete solution for bisulfite conversion followed by Whole Genome Bisulfite Sequencing (WGBS) library preparation.
- **Low Input:** Accommodates ultra-low DNA input (down to 10 pg) and is compatible with FFPE samples.
- **Simple:** Ligation- and gel-free workflow can be completed in a few hours.

Description:

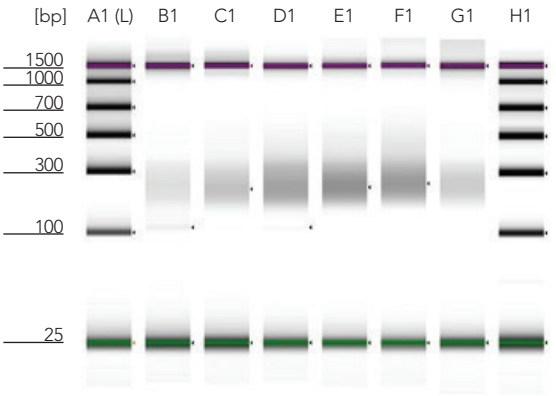
The Pico Methyl-Seq™ Library Prep Kit provides a streamlined workflow for making WGBS libraries. Input DNA is randomly fragmented during the initial bisulfite treatment step followed by three rounds of amplification with uniquely designed primers. The procedure can accommodate as little as 10 pg input DNA (including that derived from FFPE samples), making it ideal for methylation analysis of precious, limited, and target-enriched samples.

Streamlined, Innovative Workflow



Pico Methyl-Seq™ libraries ready for sequencing.

High-quality Library Preparation



Agilent 2200 TapeStation® D1K gel of libraries prepared (from B1-G1) using 10 pg, 20 pg, 100 pg, 1 ng, 10 ng, and 100 ng, respectively.

Product	Cat. No.	Size
Pico Methyl-Seq™ Library Prep Kit	D5455 D5456	10 preps 25 preps

Zymo-Seq ATAC Library Kit

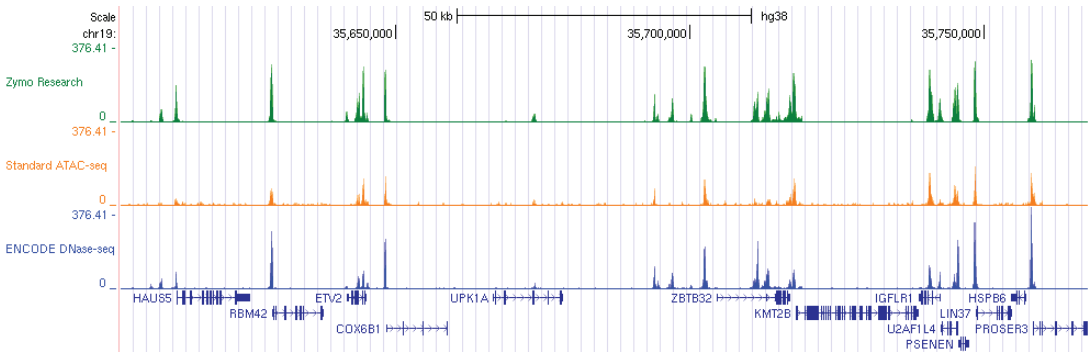
Highlights:

- **Ready to Use:** Preassembled buffers allow for lightning-fast library preparation in as little as 4 hours without compromising quality.
- **Improved Performance:** Prepare libraries with 7x less mitochondrial contamination, saving reads and increasing sequencing depth.
- **Outstanding Consistency:** Produce highly correlated replicates from both fresh and frozen samples.

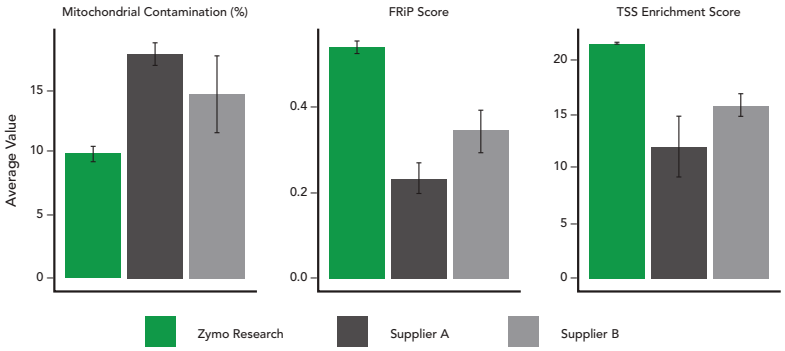
Description:

The Zymo-Seq ATAC Library Kit offers a streamlined Assay for Transposase Accessible Chromatin with Sequencing (ATAC-Seq) library preparation for mammalian cells and tissues. This all-in-one kit simplifies the process, enabling ATAC-Seq libraries from as few as 50,000 cells in just 4 hours with minimal hands-on time. First gentle cell lysis separates nuclei for collection while discarding contaminating mitochondrial DNA. The transposase, preloaded with Illumina adaptors, rapidly fragments and tags open chromatin regions in a single reaction. The resulting tagmented DNA is then indexed and PCR-amplified using the UDI Tag Primer Set, producing high-quality libraries ready for Illumina instrument sequencing.

Highest Quality Libraries



Browser tracks depicting GM12878 ATAC-seq assay using the Zymo-Seq ATAC Library Kit. Peaks overlap at the same sites identified by DNase-seq in the ENCODE project as well as the standard ATAC-seq protocol showing both quality and consistency.



Best Quality Measures

Zymo-Seq ATAC Library Kit stands ahead of its peers in common ATAC-seq quality measures, with the lowest mitochondrial read contamination, highest FRiP scores, and highest TSS enrichment scores.

Product	Cat. No.	Size
Zymo-Seq ATAC Library Kit	D5458	12 preps

Zymo-Seq™ SPLAT DNA Library Kit

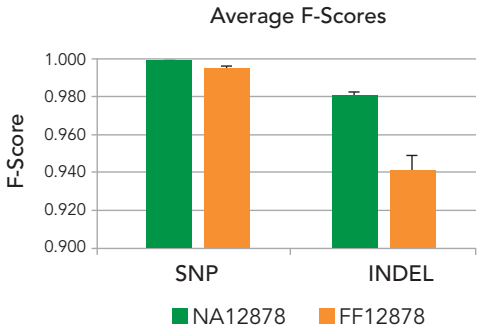
Highlights:

- **Novel Solution for Fragmentomics Analysis:** Powered by Splinted Ligation Adapter Tagging (SPLAT) technology, the kit precisely captures the true fragment ends of cell-free DNA, enabling accurate and high-fidelity analysis of fragmentation patterns.
- **Versatile Sample Handling:** The kit supports a wide range of sample types, including cell-free DNA (cfDNA), FFPE-derived DNA, and genomic DNA, while ensuring true end ligation for reliable and precise genomic data across diverse research applications.
- **Efficient Workflow:** The two-step workflow allows for the preparation of DNA samples into sequencing-ready libraries in as little as three hours, significantly speeding up the research process.

High quality SNP and INDEL performance

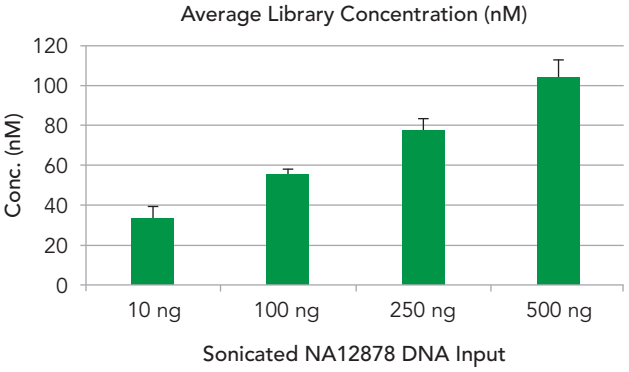
PrecisionFDA Truth Challenge

Sample Source	SNP			INDEL		
	Recall	Precision	F-Score	Recall	Precision	F-Score
NA12878	99.80%	99.95%	**0.999**	98.38%	98.22%	**0.983**
NA12878	99.83%	99.94%	**0.999**	97.99%	97.84%	**0.979**
NA12878	99.88%	99.95%	**0.999**	98.31%	98.67%	**0.980**
FF12878	99.38%	99.87%	**0.996**	95.08%	95.01%	**0.950**
FF12878	99.21%	99.85%	**0.995**	94.30%	94.17%	**0.942**
FF12878	99.10%	99.79%	**0.994**	92.97%	92.34%	**0.932**



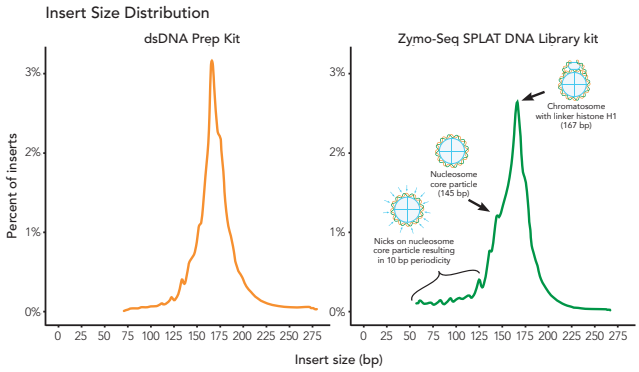
Zymo-Seq™ SPLAT DNA Library Kit produces reliable SNP and INDEL calling according to the Precision FDA Truth Challenge guidelines. Zymo-Seq™ SPLAT DNA Libraries were prepared from both genomic (NA12878) and FFPE-derived (FF12878) HG001 genome reference DNA samples. Variant calling was performed using DeepVariant. The accuracy metrics for SNP and INDEL detection were evaluated following the Precision FDA Truth Challenge workflow, comparing the results to the ground truth reference data. Overall performance of the Zymo-Seq™ SPLAT libraries shows superior results across both genomic and FFPE-derived DNA input.

High Library Yield Across Inputs



The highly optimized SPLAT library preparation facilitates abundant library across a large range of inputs. Libraries were prepared from 10 ng, 100 ng, 250 ng, and 500 ng from sonicated NA12878 genomic DNA in triplicate. The 10 ng inputs were amplified with 6 cycles of PCR, the 100 ng inputs were amplified with 5 cycles of PCR, and the 250 ng & 500 ng inputs were amplified with 4 cycles of PCR.

True-End Analysis in cfDNA



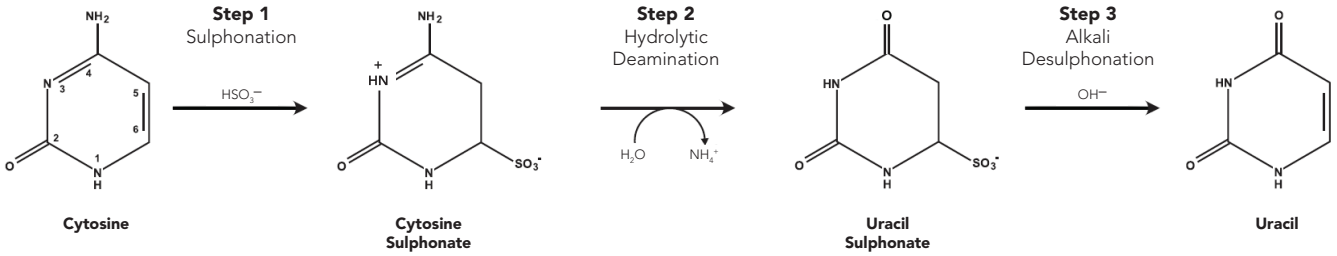
The Zymo-Seq™ SPLAT DNA Library Kit reveals more DNA fragment information than dsDNA library preparation methods. Libraries were prepared from 10 ng of cfDNA derived from a plasma donor with colorectal cancer in duplicate using the Zymo-Seq™ SPLAT DNA Library kit and a third-party dsDNA library prep kit. The libraries prepared with the unique single-stranded SPLAT approach captured enriched short cfDNA fragments compared to the dsDNA library prep method. These smaller fragment peaks, exhibiting a 10 bp periodicity, potentially correlate with nicked nucleosomal DNA.

Product	Cat. No.	Size
Zymo-Seq™ SPLAT DNA Library Kit	D5464	12 preps

Technology Overview: Bisulfite Treatment

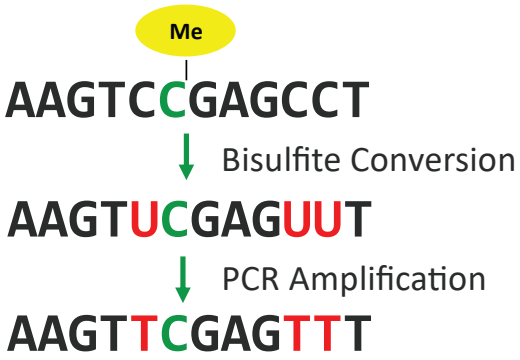
- **The Most Cited Bisulfite Reagents for Clinical Applications**
- **Conversion Workflows in as Little as 1.5 Hours with Conversion Efficiency > 99%**
- **For *in vitro* diagnostic, CE-IVD Certification**
- **Automation Ready**
- **Optimized Conditions for Many Clinical Sample Types, Including Cell-Free DNA, FFPE, Blood**

Widely considered to be the "gold standard" for the analysis of DNA methylation, bisulfite treatment works by converting unmodified cytosines to uracils in a chemical reaction. Methylated cytosines remain protected from the conversion, allowing for downstream analysis to distinguish between these epigenetic modifications present. Sequence analysis post-treatment provides site-specific information on DNA modifications across the genome. This site specificity can be accomplished by PCR, hybridization, methylation-specific PCR, NGS, and more.

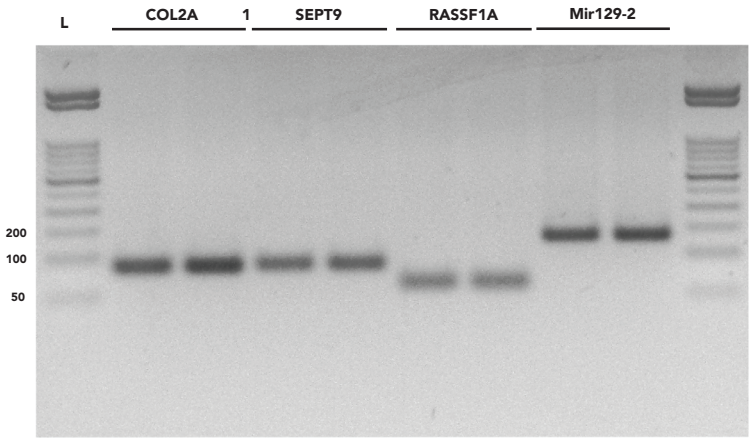


Bilsufite Technology from Zymo Research

The EZ DNA Methylation™ family of kits from Zymo Research remain the most reliable as well as the most cited technologies available for bisulfite conversion and recommended by most downstream DNA methylation analysis platforms. These kits have always pushed the limits of epigenetic innovation, from being the first methylation kit to offer on-column desulphonation to reducing conversion time down to as little as 1.5 hours. The latest EZ DNA Methylation™- Lightning kits have been specifically engineered for improved performance for fragmented clinical samples, such as cell free DNA and FFPE. MagBead based bisulfite kits offer ready to use scripts for most automated liquid handlers to achieve hight-throughput sample processing.



PCR-Ready Bisulfite DNA from FFPE



DNA isolated from a non-small-cell lung cancer (NSCLC) FFPE block was bisulfite converted using the EZ DNA Methylation-Lightning Kit, then CR amplified with methylation-specific primers for cancer biomarkers SEPT9, RASSF1A, Mir129-2, and control primer COL2A1. L=50bp marker

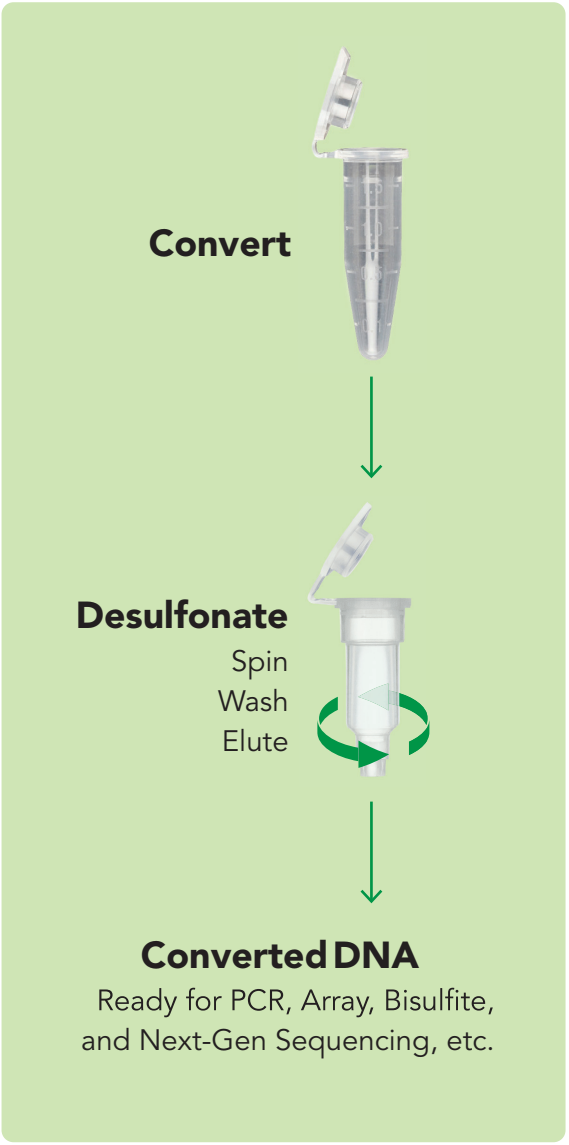
The Evolution of Zymo Research's EZ DNA Methylation™ Kits

Zymo Research's bisulfite technology emerged from the necessity for a reliable method to study DNA methylation. Developed to accurately detect methylation status in DNA, the bisulfite chemistry converts unmethylated cytosines to uracil while preserving methylated cytosines. This treatment, enables researchers to differentiate between methylated and non-methylated cytosines through subsequent sequencing or analysis.

A standout feature of Zymo Research's technology is its pioneering use of a spin column-based desulphonation/ purification step. This innovative approach served to make the workflow both efficient and convenient. Additionally, it reduced the risk of sample loss and contamination, making it the preferred choice for epigenetic researchers.

Widely accepted as the gold standard in DNA methylation analysis, the EZ DNA Methylation line of bisulfite kits are distinguished by their high conversion efficiency, reliability, and reproducibility. Rigorous testing and optimization ensure consistent and accurate detection of DNA methylation patterns.

Continuously evolving based on user feedback and technological advancements, the EZ DNA Methylation-Lightning™ Kits are the pinnacle for investigating DNA methylation and deciphering epigenetic regulation in health and disease.



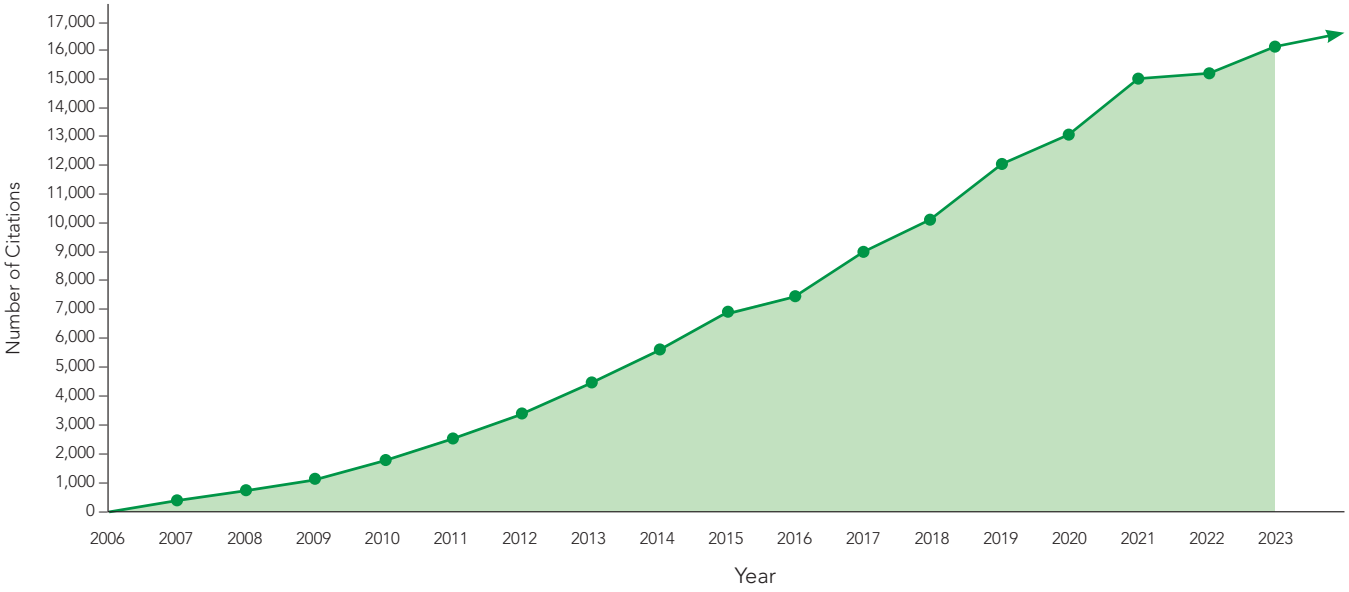
Product Selection Guide: EZ DNA Methylation™

All bisulfite conversion kits are available in spin-column, 96-well, and MagBead formats. These innovative bisulfite conversion kits feature streamlined workflows, ≥ 99% conversion rates, automation friendly options, and low elution volumes for concentrated, bisulfite-converted DNA. All MagBead versions are automation ready with free scripts available!

	EZ DNA Methylation-Lightning™*	EZ DNA Methylation-Direct™	EZ DNA Methylation-Gold™	EZ DNA Methylation™
	Rapid workflow, most gentle chemistry	Use directly with cells and tissues	Classic bisulfite conversion	Classic bisulfite conversion
Which kit is right for me?	Best for new users and Illumina Array users	Best for working with cells/tissue as direct input, and validated third party kits	Best for legacy users and users working with validated third-party kits	Best for legacy users and Illumina Array users
Conversion Efficiency	> 99.5%	> 99.5%	> 99%	> 99%
Input	100 pg – 2 µg of DNA	DNA (≥ 50 pg), cells (≥ 10), blood, tissue, FFPE	500 pg – 2 µg of DNA	500 pg – 2 µg of DNA
Processing Time	1.5 hr	4 hr	3 hr	12-16 hr
Validated For	Illumina MethylationEPIC Array (MagBead format) (D5046, D5047, D5049)	IDT xGen Adaptase module for Single-Cell Methyl-Seq	Agilent SureSelect MethylSeq, IDT xGen™ Methylation-Sequencing	Illumina MethylationEPIC Array (All formats) (D5001, D5002, D5004)

*CE IVD

The Most-Cited Technologies for DNAMethylation Analysis & Detection



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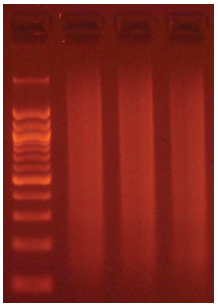
Bisulfite Conversion Resources

Learn More About Bisulfite Conversion

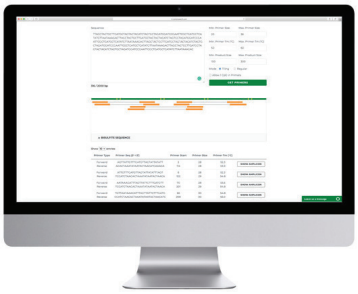
- Visualizing Bisulfite-Converted DNA
- Quantification of Bisulfite-Converted DNA
- PCR of Bisulfite Converted DNA



Scan to Learn More
https://www.zymoresearch.com/pages/bisulfite-beginner-guide

Bisulfite Primer Seeker

This program streamlines the tedious process of bisulfite primer design. This program will help you design primers in particularly CG-rich sequences and will provide you with multiple options for amplicons that span different regions within your sequence.



Scan to Use the Free Bisulfite Primer Design Tool
https://www.zymoresearch.com/pages/bisulfite-primer-seeker

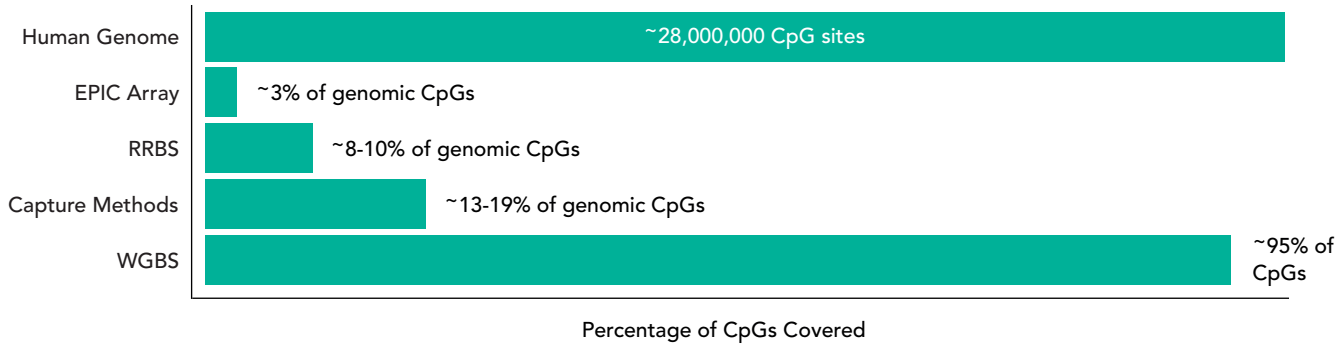
A Comprehensive Guide for Illumina Methylation Arrays

- Troubleshooting & best practice guidelines
- Bisulfite kit selection guide from Illumina
- Automated bisulfite conversion for Infinium™ Methylation BeadChips

A Quick Guide for DNA Methylation Profiling with NGS-based Methods

Determine the best whole-genome methylation analysis method based on the desired CpG coverage.

Whole-Genome Bisulfite Sequencing (WGBS) Expands the Frontiers of Genomic Medicine



Scan to View
https://www.zymoresearch.com/pages/what-is-dna-methylation

Scan to Read the App Note:
High-accuracy Next-Gen Sequencing with the NovaSeq™ Series

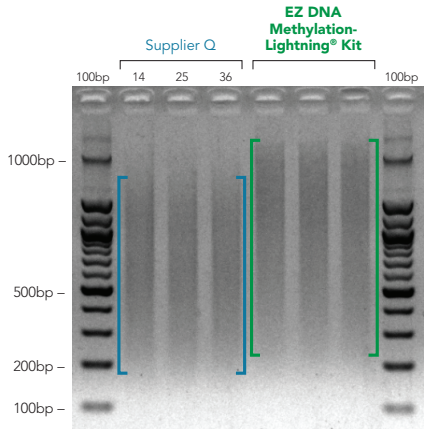
EZ DNA Methylation-Lightning™ Kits

Highlights:

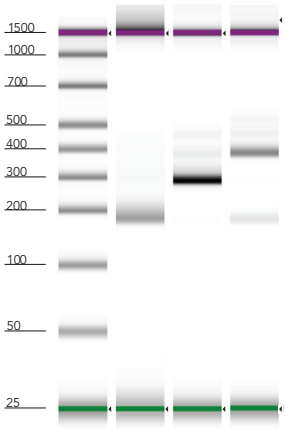
- **Streamlined Process:** Ready-to-use conversion reagent is added directly to DNA. Purified bisulfite converted DNA in <1.5 hours.
- **High-Quality:** Bisulfite-converted DNA has > 99.5% conversion efficiency with reduced fragmentation.
- **NGS-Ready:** Low DNA input requirement makes it ideal for preparing whole genome or targeted enrichment bisulfite libraries for methylation analysis.

Description:

The EZ DNA Methylation-Lightning™ Kits feature the fastest bisulfite conversion methodology resulting in fully converted DNA with reduced fragmentation and more efficient PCR amplification. The bisulfite converted DNA is ideal for consistent, high-quality DNA methylation analyses such as PCR, MSP, array, bisulfite and NGS. The Lightning technology is optimized for a broad range of samples including fragmented or degraded inputs.



The EZ DNA Methylation-Lightning™ Kit yields more intact DNA after bisulfite conversion than the comparable kit from Supplier Q.



Recovery of Small Fragments

Bisulfite converted libraries of small 100, 200, and 300 bp DNA fragments were successfully recovered and amplified by PCR. Libraries were analyzed using the Agilent 4200 TapeStation® system.

Product	Cat. No.	Size	Specifications	Uses
EZ DNA Methylation-Lightning™ Kit SK	D5030T D5030/D5030-E* D5031/D5031-E*	10 Rxns. 50 Rxns. 200 Rxns.	Input: 100 pg to 2 µg of purified DNA Conversion Efficiency: > 99.5% Format: Spin Column Elution Volume: ≥ 10 µl DNA Recovery: > 80% Bisulfite Conversion Time: 1.5 hours	Rapid bisulfite treatment; Rapid column/plate/bead desulphonation
EZ-96 DNA Methylation-Lightning™ Kit (Shallow-Well)	D5032	2 x 96 Rxns.	Input: 100 pg to 2 µg of purified DNA Conversion Efficiency: > 99.5% Format: 96-Well Elution Volume (shallow-well): ≥ 30 µl Elution Volume (deep-well): ≥ 15 µl DNA Recovery: > 70% Bisulfite Conversion Time: 1.5 hours	
EZ-96 DNA Methylation-Lightning™ Kit (Deep-Well)	D5033	2 x 96 Rxns.		
EZ-96 DNA Methylation-Lightning™ MagPrep Kit	D5046/D5046-E* D5047/D5047-E*	4 x 96 Rxns. 8 x 96 Rxns.	Input: 100 pg to 2 µg of purified DNA Conversion Efficiency: > 99.5% Format: Magnetic Beads Elution Volume: ≥ 25 µl DNA Recovery: > 70% Bisulfite Conversion Time: 1.5 hours	
EZ-96 DNA Methylation-Lightning™ Automation Kit	D5049	96 preps	Input: 100 pg to 2 µg of purified DNA Conversion Efficiency: > 99.5%Format: Magnetic Beads Elution Volume: 25µl DNA Recovery: > 80% Bisulfite Conversion Time: 1.5 hours	Rapid bisulfite treatment for automated workflows

SK - Sample Kit Available *CE IVD

EZ DNA Methylation-Direct™ Kits

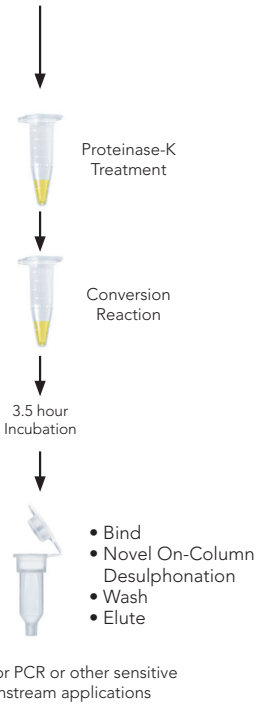
Highlights:

- **No Purification Necessary:** Complete bisulfite conversion of DNA directly from blood, soft tissue, cells, FFPE, and LCM samples.
- **Low-Input:** Compatible with small sample inputs, as few as 10 cells or 50 pg DNA.
- **High-Quality DNA**: Converted DNA is ready for PCR and NGS.

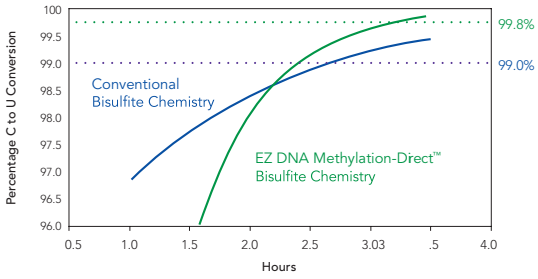
Description:

The EZ DNA Methylation-Direct™ Kit is a further refinement of our popular EZ DNA Methylation™ and EZ DNA Methylation-Gold™ kits. The EZ DNA Methylation-Direct™ Kit features reliable and complete bisulfite conversion of DNA directly from blood, tissue, and cells without the prerequisite for DNA purification. The increased sensitivity of these kits make it possible to amplify bisulfite-converted DNA from as few as 10 cells or 50 pg DNA. Many single-cell applications and DNA methylation methods have been developed based on EZ DNA Methylation-Direct™ chemistry.

Cells, Blood, Tissue, FFPE, etc.



EZ DNA Methylation-Direct™ Bisulfite Chemistry Significantly Improves C to U Conversion Kinetics



EZ DNA Methylation-Direct™ Kit bisulfite chemistry significantly improves C to U conversion kinetics. DNA was converted using either EZ DNA Methylation-Direct™ or conventional bisulfite chemistries. Recovered DNA was amplified by PCR, then cloned. Sequences from individual clones were analyzed and quantitated. This data shows that EZ DNA Methylation-Direct™ bisulfite chemistry improves the rate and extent (> 99.8%) of C to U conversion of DNA compared to conventional bisulfite chemistry.

Product	Cat. No.	Size	Specifications	Uses
EZ DNA Methylation-Direct™ Kit	D5020 D5021	50 Rxns. 200 Rxns.	Input: DNA, Cells, Blood, Tissue, FFPE (as few as 10 cells or 50 pg) Conversion Efficiency: > 99.5% Format: Spin-Column Elution Volume: ≥ 10 µl DNA Recovery: > 80% Bisulfite Conversion Time: 4 hours	Sample DNA digestion; Bisulfite treatment; Rapid column/plate/ bead desulphonation
EZ-96 DNA Methylation-Direct™ Kit (Shallow-Well)	D5022	2 x 96 Rxns.	Input: DNA, Cells, Blood, Tissue, FFPE (as few as 10 cells or 50 pg) Conversion Efficiency: > 99.5% Format: 96-Well Elution Volume (shallow-well): ≥ 30 µl Elution Volume (deep-well): ≥ 15 µl DNA Recovery: > 70% Bisulfite Conversion Time: 4 hours	
EZ-96 DNA Methylation-Direct™ Kit (Deep-Well)	D5023	2 x 96 Rxns.		
EZ-96 DNA Methylation-Direct™ MagPrep Kit	D5044 D5045	4 x 96 Rxns. 8 x 96 Rxns.	Input: DNA, Cells, Blood, Tissue, FFPE (as few as 10 cells or 50 pg) Conversion Efficiency: > 99.5% Format: Magnetic Beads Elution Volume: ≥ 25 µl DNA Recovery: > 70% Bisulfite Conversion Time: 4 hours	

EZ DNA Methylation™ MagPrep Series

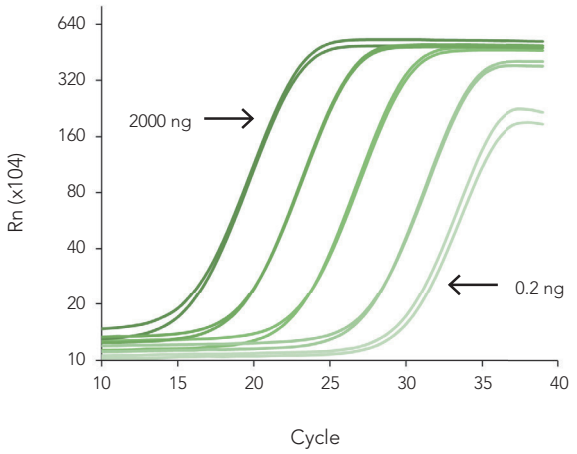
Highlights:

- **Scalable:** Streamlined workflow adaptable for manual or automated processing.
- **Reliable:** Incorporates citation-leading bisulfite chemistries with low-elution bead-based purification to recover > 50 pg of DNA for methylation analysis.
- **NGS-ready:** Purified bisulfite converted DNA is ideal for library preparation, methylation-sensitive PCR assays, microarrays, etc.

Description:

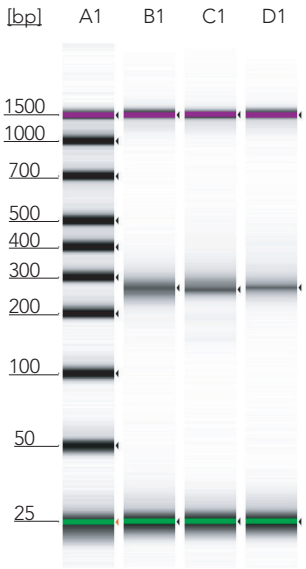
The EZ DNA Methylation™ MagPrep pairs our citation-leading bisulfite chemistries with magnetic bead-based purification for high-throughput and automated methylation analysis. The DNA undergoes bisulfite treatment to convert all cytosine into uracil. Desulphonation and clean-up of the converted DNA is performed while bound to the MagBinding Beads. The kit is designed to reduce template degradation and minimize DNA loss during treatment and clean-up, while ensuring complete conversion of the DNA. Purified, converted DNA is ideal for downstream analyses including NGS, PCR amplification, endonuclease digestion, microarrays, etc.

Linear Recovery of Bisulfite DNA



The EZ-96 DNA Methylation-Lightning™ MagPrep is compatible with a range of DNA inputs and has a linear recovery (R2 = 0.9977). A 10-fold dilution series of human genomic DNA, ranging from 2000 ng to 0.2 ng, was bisulfite converted, and recovery was assessed by qPCR targeting the DAPK1 region (n = 2).

Consistent Library Preparation



Three pools of targeted bisulfite sequencing libraries were analyzed on the Agilent 2200 Tapestation® HSD1000. Each pool contains over 35 samples that were individually bisulfite converted using the DNA Methylation-Lightning™ MagPrep followed by library preparation.

Product	Cat. No.	Size
EZ-96 DNA Methylation™ MagPrep	D5040 D5041	4 x 96 Rxns. 8 x 96 Rxns.
EZ-96 DNA Methylation-Gold™ MagPrep	D5042 D5043	4 x 96 Rxns. 8 x 96 Rxns.
EZ-96 DNA Methylation-Direct™ MagPrep	D5044 D5045	4 x 96 Rxns. 8 x 96 Rxns
EZ-96 DNA Methylation-Lightning™ MagPrep	D5046/D5046-E* D5047/D5047-E*	4 x 96 Rxns. 8 x 96 Rxns.

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OneStep PLUS qMethyl™ PCR Kit

Highlights:

- Single step, bisulfite-free DNA methylation analysis.
- Includes reagents and controls for quantitative detection and reliable performance.
- Ideal for rapid screening of single and multi-locus DNA methylation.

Description:

The OneStep PLUS qMethyl™ PCR Kit is intended for the quantification of DNA methylation at custom-selected genomic regions. The same DNA sample is analyzed in parallel with the Test PreMix and the Reference PreMix. The Test PreMix contains enzymes that selectively degrade (digest) unmethylated DNA while leaving methylated DNA intact. Only methylated DNA will be amplified in the "Test Reaction". The Reference PreMix does not contain those enzymes, therefore both methylated and unmethylated DNA are amplified in the "Reference Reaction". The difference in Cycle threshold (Ct) values between the Reference and Test reactions is used to calculate the percentage of DNA methylation at the selected genomic region.

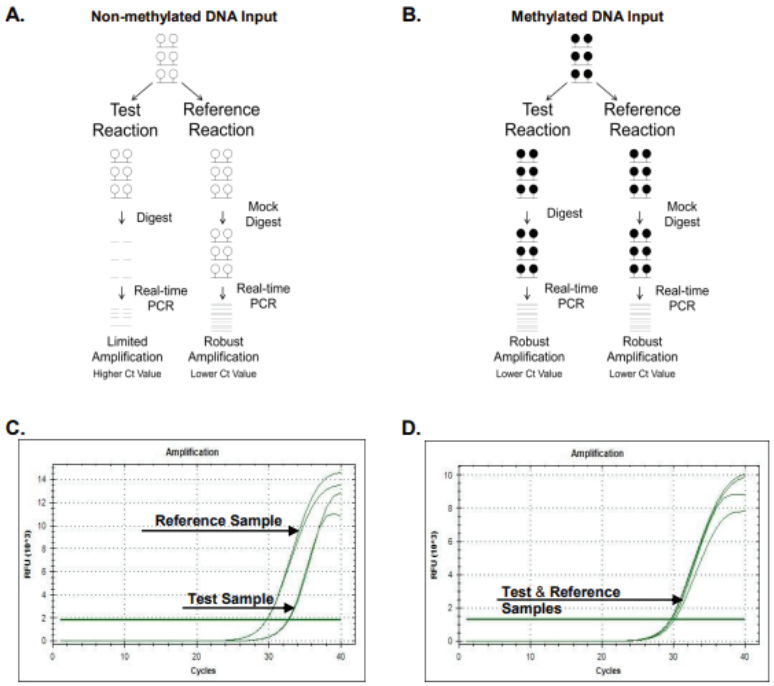


Figure 1. A and B are the schematic representation of OneStep PLUS qMethyl™ PCR test for non-methylated DNA and methylated DNA, respectively. Unmethylated and methylated CpG sites are represented by white and black circles, respectively. In both schemes the DNA is tested in two reactions: a Test Reaction, in which unmethylated DNA is digested, and a Reference Reaction, in which no digestion occurs. Quantitative PCR is carried out immediately after the digestion step. C and D are examples of amplification curves obtained from Human Non-Methylated & Methylated DNA Standards, respectively. The higher Ct values for the Test Reaction compared to the Reference reaction in figure C indicates that the unmethylated DNA fraction has been degraded during the digestion step.

Product	Cat. No.	Size	Specifications	Uses
OneStep PLUS qMethyl™ PCR Kit	D5312	96 Rxns.	Input: Optimal DNA input is 20 ng in 5 µl. Range validated 5 ng – 1 µg (in 5 µl) Detection Dye: Cyto 9. Assay is flexible with other dyes and TaqMan probes. Compatible Thermal Cyclers: Bio-rad CFX-96™, CFX Opus 96™, QuantStudio qPCR Systems, Roche LightCycler 480®, or similar. Processing Time: Optimal 14 hours.	Bisulfite-free DNA methylation analysis; Rapid screening of multiple loci or single locus across multiple samples

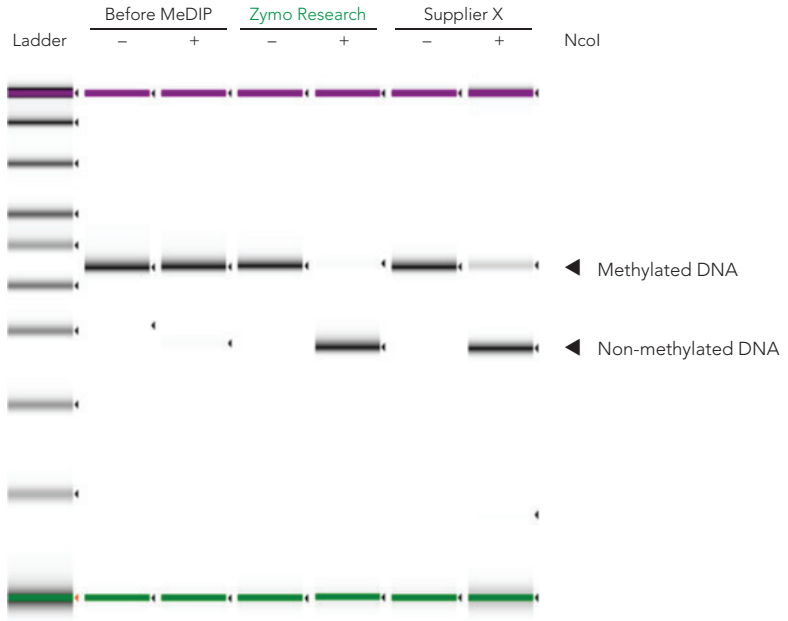
Methylated-DNA IP Kit

Highlights:

- **Reliable:** Robust enrichment & imm unoprecipitation of 5-mC containing DNA.
- **Streamlined:** Includes a highly specific anti-5-methylcytosine monoclonal antibody for defined, reproducible results.
- **High-Quality:** Eluted, ultra-pure DNA is ideal for use in subsequent molecular based analyses (e.g., assembling genomic libraries and determining genome-wide methylation status).

Description:

The Methylated-DNA IP Kit is designed for enrichment of 5-mC-containing DNA from any pool of fragmented genomic DNA for use in genome-wide methylation analysis. It features a highly specific Anti-5-Methylcytosine Monoclonal Antibody for the immunoprecipitation of methylated DNA in only a few hours. This kit is capable of achieving over one hundred-fold enrichment of methylated DNA vs. non-methylated DNA. Recovered DNA is suitable for many downstream applications to analyze genome-wide DNA methylation including PCR, bisulfite treatment, whole-genome amplification, ultra-deep sequencing, and microarray. Control DNA and primers are included to monitor the success of the assay.



Methylated DNA is efficiently enriched using the 5-Methylcytosine antibody. Control DNA comprised of a mixture of methylated and non-methylated was immunoprecipitated using mouse Anti-5-Methylcytosine antibody from Zymo Research or Supplier X. The methylated DNA contains point a mutation that introduces an NcoI restriction site. After immunoprecipitation of the mixture, the region of DNA containing the restriction site was amplified by PCR, digested with NcoI, and visualized using the Agilent 2200 Tapestation®. Non-methylated DNA remains un-cut, whereas the methylated DNA is cut by NcoI. The image above demonstrates specific enrichment of methylated versus non-methylated DNA by the Anti-5-Methylcytosine from Zymo compared to Supplier X.

Product	Cat. No.	Size	Specifications	Uses
Anti-5-Methylcytosine Monoclonal Antibody (Clone 7D21)	A3002-15 A3002-30 A3002-50 A3002-200	15 µl 30 µl 50 µl 200 µl	Isotype: IgG1 Concentration: 5 µg/µl Buffer: PBS (pH 7.4) 0.05% Sodium Azide Short Term Storage: 4°C Longe Term Storage: -80°C	Immunoprecipitation of methylated DNA; ELISA; Immunoblotting; Immunofluorescence
Methylated-DNA IP Kit	D5101	10 Rxns.	Format: Magnetic Beads Optimal DNA Input: 50 - 500 ng Elution Volume: 10 µl Enrichment Factor: > 100 fold Processing Time: 4 hours	Immunoprecipitation of methylated DNA; PCR; Sequencing

Choose Your Epigenetic Standards

Industry-leading DNA methylation standards for seamless quantification. Assess your DNA methylation workflow today!

DNA methylation standards can be used for optimizing a variety of methylation assays or can serve as positive and negative controls to validate established workflows. Standards are also ideal substitutes for precious DNA or problematic sample types when troubleshooting or testing experimental conditions. DNA methylation standards have utility in various applications including bisulfite sequencing using NGS technologies, methylation-specific PCR (MSP), and methylation-sensitive restriction enzyme (MSRE) assays. Integrating established controls is also key for clinical workflows and during all assay calibrations.

Choose from a range of DNA standards that can be used for optimization or quality control of various methylation assays.

Catalog No.	Product	0% and 100% Methylation Controls	Bisulfite PCR	Methylation-Specific PCR	NGS Library Optimization	*In Situ* Control	Methylation Assay Calibration
D5011	Universal Methylated Human DNA Standard		X		X		
D5012	Universal Methylated Mouse DNA Standard		X		X		
D5013	Human Methylated & Non-Methylated (WGA) DNA Set	X	X	X			
D5014	Human Methylated & Non-methylated DNA set	X	X	X	X		X
D5015	Bisulfite-Converted Universal Methylated Human DNA Standard		X				
D5016	*E. Coli* Non-Methylated Genomic DNA		X		X	X	X
D5017	Methylated & Non-methylated pUC19 DNA Set	X	X			X	X
D5018	Human Matched DNA Set		X		X		X
D5019	Mouse 5-hmC & 5-mC DNA Set		X		X		X
D5405	5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set	X				X	
D5500	Zymo-Seq Methyl Spike-in Control	X			X	X	X

Scan to Learn More About Controls for Methylation Assays
https://www.zymoresearch.com/blogs/blog/controls-for-dna-methylation-assays

Human Methylated & Non-Methylated DNA Set

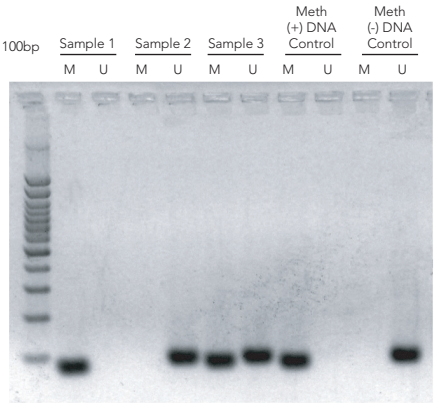
Highlights:

- **Ideal Positive and Negative Controls:** DNA standards, purified from HCT116 DKO cell line, for use as positive and negative controls in methylation-detection applications including bisulfite PCR (BSP) and methylation-specific PCR (MSP) experiments.
- **Standard Curve Generation:** Completely methylated and non-methylated DNA can be mixed together in various proportions to generate a standard curve for suitable quantitation of DNA methylation in experimental samples.
- **Convenient:** Provided with control primers to amplify a fragment of DNA after bisulfite conversion.

Description:

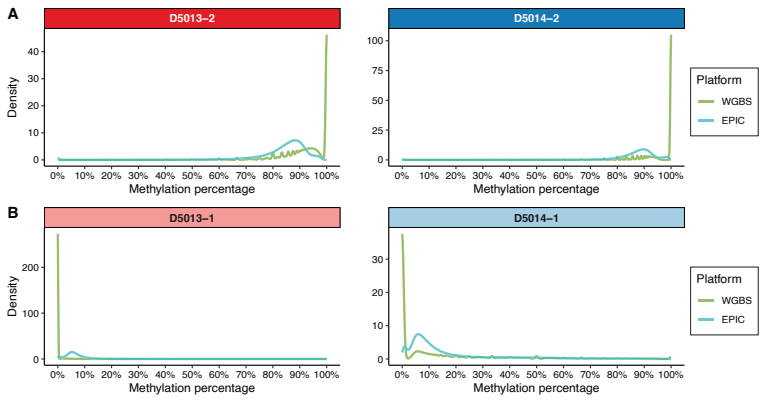
The Human Methylated & Non-Methylated DNA Set consists of two control DNAs (a CpG methylated human DNA standard and a non-methylated human DNA standard). These DNA standards come in both genomic (D5014) and whole-genome amplified (D5013) versions, with a set of specifically designed primers that can be used in conjunction with the EZ DNA Methylation™ family kits (page 192-196). These DNA sets can be included as a positive and negative control to assess the efficiency of bisulfite-mediated conversion of DNA.

The non-methylated human DNA is purified from the HCT116 DKO (double knock-out) cell line, which contains genetic knockouts of both DNA methyltransferases DNMT1 (-/-) and DNMT3b (-/-). The methylated DNA standard is purified HCT116 DKO DNA that has been enzymatically methylated at CpG sites.



Example MSP experiment using MSP designed primers for RASSF1. Sample 1 is positive for a Methylated Template. Sample 2 is positive for a Non-Methylated Template and Sample 3 contains Methylated and Non-Methylated Templates. MSP experiment also shows proper controls: Meth (+) DNA Control corresponds to the Human Methylated DNA standard (D5014-2) and the Meth (-) DNA Control corresponds to the Human Non-methylated DNA standard (D5014-1). 2% Agarose Gel, 130V for 35 mins. M = Methylated specific primers, U = Non-Methylated specific primers

Methylation Distribution and Agreement



Methylation estimates across the genome. Each platform's methylation percentages were collected for all loci represented and compared. Density was calculated for each platform from the methylation percentages for the D5013 and D5014 methylation standards. (A) methylated standards D5013-2 (left) D5014-2 (right) (B)non-methylated standards, D5013-1 (left) D5014-1 (right)

(Cited from: X. Yang, R. Yancey, et al. Assessing HiFi Long Read Sequencing versus Whole Genome Bisulfite Sequencing and Methylation EPIC BeadChip Array: A Comparative Analysis Utilizing DNA Methylation Standards.; (Abstract:PB2225). Presented at the Annual Meeting of The American Society of Human Genetics, Nov 1st 2023, Washington D.C.)

Product	Cat. No.	Size	Specifications	Uses
Human Methylated & Non-Methylated (WGA) DNA Set	D5013	5 µg/20 µl	Format: HCT116 DKO Genomic DNA Concentration: 250 ng/µl	Control for bisulfite conversion; DNA methylation quantitation
Human Methylated & Non-methylated DNA Set	D5014	5 µg/20 µl		

Zymo-Seq Methyl Spike-in Control

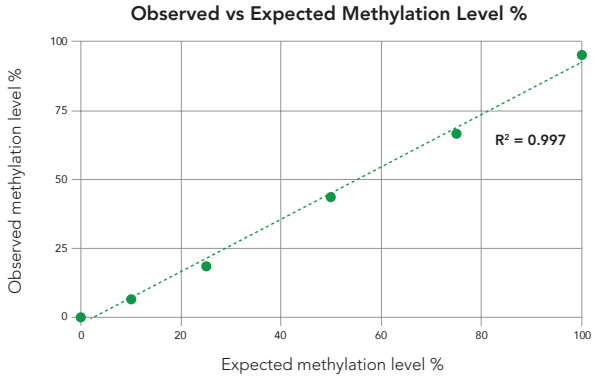
Highlights:

- Ensures reliable calculation of bisulfite conversion efficiency post library prep.
- Six unique amplicons with 0, 10, 25, 50, 75, and 100% methylation levels allow for a standard curve and robust data normalization.
- Compatible with various species (except for *E. coli*) and bisulfite sequencing library preparation methods.

Description:

Zymo-Seq Methyl Spike-in Control is comprised of six unique double-stranded synthetic amplicons (180-200 bp) with distinct sequences derived from the *E. coli* K12 genome, and each amplicon represents a different CpG methylation level ranging from 0% to 100%. This control serves as an *in situ* control for NGS library preparations, providing an unbiased way of calculating the efficiency of the bisulfite conversion reaction. It can also be used to validate bioinformatics pipeline calibration by demonstrating a strong correlation between observed and expected methylation levels of the amplicons.

Accurate Methylation Detection



The observed methylation levels of a Zymo-Seq Methyl Spike-in Control, exhibit a high correlation with the expected methylation level when used with the Zymo-Seq WGBS Library Kit (D5465). Bioinformatic analysis using the reference control with known methylation values ensures high-quality data for each individual sample.

True Bisulfite Conversion Efficiency in Non-CpG Context

Species	Sample gDNA	Sample DNA with Zymo-Seq Methyl Spike-in Control
Cotton	81%	99%
Soybean	89%	99%
Arabidopsis thaliana	97%	99%
Cattle	99%	99%
Human	99%	99%

Bisulfite conversion efficiency in non-CpG context from various species was measured using the sample gDNA with and without Zymo-Seq Methyl Spike-in Control. Utilizing the Zymo-Seq Methyl Spike-In Control resulted in improved accuracy in calculations, especially for non-traditional organisms that have methylation in non-CpG context.

Product	Cat. No.	Size	Specifications	Uses
Zymo-Seq Methyl Spike-in Control	D5500	25 preps	Format: Double-stranded Synthetic Amplicons Derived from the *E. coli* Genome Concentration: 60 pg/µl	Control for bisulfite conversion; DNA methylation quantitation

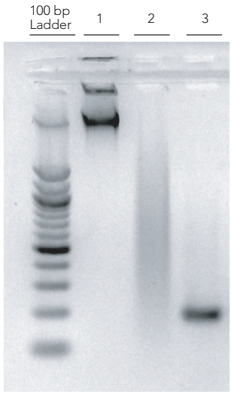
Universal Methylated DNA Standards

Highlights:

- **Ideal Highly-Methylated Controls:** Purified DNA from normal human or mouse tissue that is enzymatically methylated at all CpG sites for use as a positive control.
- **Side-by-Side Processing:** Standards can be processed in parallel with experimental samples to monitor bisulfite conversion efficiency.
- **Convenient:** Provided with control primers to amplify a fragment of DNA after bisulfite conversion.

Description:

The Universal Methylated DNA Standards are designed for use as positive controls to assess the efficiency of bisulfite-mediated conversion of DNA. The control DNAs can be assayed in parallel with samples to monitor the bisulfite conversion reaction. Each primer set has been designed to amplify a fragment of the supplied DNA following bisulfite treatment.



Assess Bisulfite Conversion Efficiency and Primer Design

Gel electrophoresis depicting genomic DNA, bisulfite-converted genomic DNA, and genomic DNA amplified with bisulfite-specific primers. Lane 1 – Input DNA: Universal Methylated Human DNA Standard (D5011). Lane 2 – Bisulfite-converted Universal Methylated Human DNA (D5011) using EZ DNA Methylation-Direct™ Kit (D5020). Lane 3 – Universal Methylated Human DNA (D5011) bisulfite converted and amplified with supplied hMLH1 control primers.

Additional Bisulfite Conversion Controls

Description:

The Bisulfite-Converted Universal Methylated Human DNA Standard is designed to be used as a control for DNA bisulfite conversion and downstream analyses including PCR, MSP, and other amplification-based assays. This DNA is identical to our Universal Methylated Human DNA Standard, but has been bisulfite-converted using the EZ DNA Methylation – Direct (D5020) kit. The primer set included with the standard has been designed and validated to amplify a segment of the bisulfite-converted DNA.

The Methylated & Non-methylated pUC19 DNA Set consists of control DNAs and a set of specifically designed primers. The set is ideal as a "spike-in" control to assess bisulfite conversion efficiency within the same reaction as the sample, or to produce known mixtures of methylated and non-methylated DNA for assay calibration.

E. coli non-methylated genomic DNA is from a Dam– and Dcm– strain (ER2925) of *E. coli*. It works perfectly as a negative control for DNA methylation analyses requiring DNA with absolutely no methylation.

Product	Cat. No.	Size	Specifications	Uses
Universal Methylated Human DNA Standard	D5011	5 µg/20 µl	Format: Male Genomic DNA Concentration: 250 ng/µl	Control for bisulfite conversion; DNA methylation quantitation
Universal Methylated Mouse DNA Standard	D5012	5 µg/20 µl		
Bisulfite-converted Universal Methylated Human DNA Standard	D5015	1 µg/50 µl	Format: Bisulfite-converted Male Genomic DNA Concentration: 20 ng/µl	
Methylated & Non-Methylated pUC19 DNA Set	D5017	20 ng/20 µl	Format: Linearized Plasmid Concentration: 1 ng/µl	
E. coli Non-Methylated Genomic DNA	D5016	1 µg/50 µl	Format: *E. coli* Genomic DNA Concentration: 250 ng/µl	

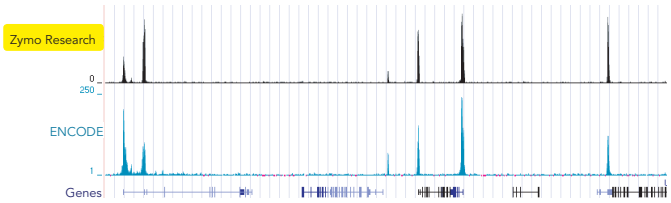
Zymo-Spin™ ChIP Kit

Highlights:

- **Simplified Workflow:** Streamlined protocol for chromatin immunoprecipitation and purification of ChIP DNA.
- **High-Quality:** Ultra-pure, concentrated ChIP DNA can be eluted in as little as 6 µl.
- **NGS-Ready:** ChIP DNA is suitable for ChIP-Seq, ChIP-qPCR, and other sensitive molecular applications.

Description:

The Zymo-Spin™ ChIP Kit from Zymo Research provides a streamlined ChIP procedure for investigating protein-DNA interactions that have been "fixed" in their natural state and can be used to effectively identify binding sites for transcription factors, co-factors, and other DNA regulatory proteins.

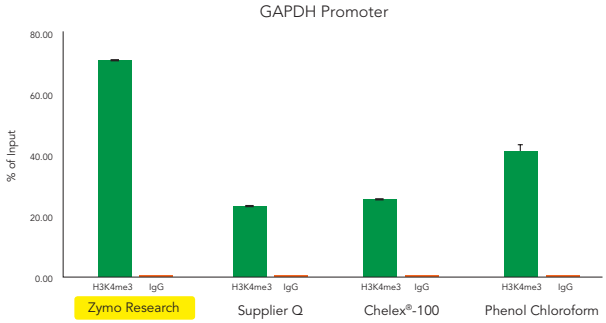


ENCODE Quality ChIP Workflow: Browser tracks depicting H3K4me3 ChIP-Seq assay using the Zymo-Spin™ ChIP Kit. Peaks overlap the same sites identified at the Broad Institute of MIT and Harvard as part of the ENCODE project.

ChIP DNA Clean & Concentrator® Kit

Highlights:

- **Fast:** Two-minute DNA clean-up from any step in a standard ChIP protocol.
- **High-Quality:** Ultra-pure, concentrated ChIP DNA can be eluted in as little as 6 µl.
- **Ready-to-Use:** DNA is ideal for PCR, arrays, DNA quantification, Southern blot analysis, sequencing, and other molecular applications.



Efficient DNA Clean-up from ChIP Protocols

ChIP DNA Purification Comparison: ChIP assays were performed with HeLa cells using ChIP-grade anti-H3K4me3 and rabbit IgG antibodies. Both total and immunoprecipitated chromatin were reverse cross-linked and recovered using either the ChIP DNA Clean & Concentrator® (included in the Zymo-Spin™ ChIP Kit), DNA recovery kit from Supplier Q, Chelex®-100 protocol or phenol-chloroform extraction. The amount of ChIP DNA was determined using qPCR with primers specific to the GAPDH promoter. ChIP DNA enrichment is graphed as % input.

Product	Cat. No.	Size	Specifications	Uses
Zymo-Spin™ ChIP Kit	D5209 D5210	10 preps 25 preps	Sample Source: Mammalian Cells	Chromatin Immunoprecipitation (ChIP)
ChIP DNA Clean & Concentrator® (Uncapped Columns	D5201	50 preps	Format: Spin-Column Elution Volume: ≥ 6 µl DNA Size Limit: 50 bp - 23 kb DNA Recovery: 50 bp - 10 kb 70-90%; > 10 kb 70% Binding Capacity: 5 µg Processing Time: 2 minutes	DNA purification from any step in a ChIP assay
ChIP DNA Clean & Concentrator® (Capped Columns)	D5205	50 preps		
ZR-96 ChIP DNA Clean & Concentrator®	D5206 D5207	2 x 96 Rxns. 4 x 96 Rxns.	Format: 96-Well Elution Volume: ≥ 10 µl DNA Size Limit: 50 bp - 23 kb DNA Recovery: 50 bp - 10 kb 70-90%; > 10 kb 70% Binding Capacity: 5 µg Processing Time: 45 minutes	

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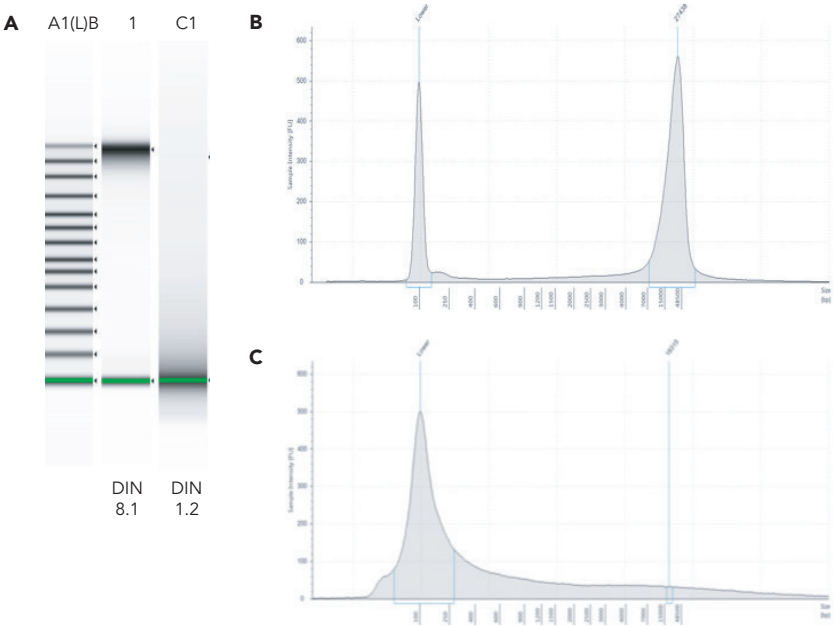
DNA Degradase™ & DNA Degradase Plus™

Highlights:

- **Fast:** One-hour, single-enzyme digestion solution to the conventional 6-16 hour multi-step enzyme digestion protocols.
- **Streamlined Workflow:** Quick, simple procedure for completely degrading DNA into individual nucleotides (DNA Degradase™) or nucleosides (DNA Degradase Plus™).
- **No Clean-Up Necessary:** Digested DNA products are immediately ready for downstream analysis by global quantitative methods including HPLC, TLC, and LC-MS.

Description:

DNA Degradase™ and DNA Degradase Plus™ are nuclease mixes that quickly and efficiently degrade DNA to its individual nucleotide or nucleoside components, respectively. DNA Degradase™ is ideal for global DNA methylation analysis, including hydroxymethylation and other demethylation intermediate products, by a number of downstream applications (i.e., LC-MS, HPLC, TLC, etc.). Digestion with the enzyme is a simple single-step procedure that works faster than other available methods.



DNA Degradase Plus™ efficiently degrades DNA. Mouse brain DNA (1 µg) was digested with 5 U of DNA Degradase Plus™ for 1 hr at 37°C and analyzed using Agilent 2200 TapeStation®. A) TapeStation gel image (A1- genomic ladder, B1- control DNA, C1- DNA Degradase Plus™ digested DNA). Electropherogram of control DNA (B) and DNA Degradase Plus™ digested DNA (C).

Product	Cat. No.	Size	Specifications	Uses
DNA Degradase™	E2016 E2017	500 U 2000 U	Enzyme Concentration: 10 U/ µl Storage: -20°C Inactivation: 70°C for 20 minutes Standard Reaction Time: 1 hour One unit (U) is defined as the amount of enzyme required to degrade 1 µg of DNA in a total reaction volume of 25 µl for 1 hour at 37°C.	Complete digestion of DNA into individual nucleotide/nucleoside components
DNA Degradase Plus™	E2020 E2021	250 U 1000 U	Enzyme Concentration: 5 U/ µl Storage: -20°C Inactivation: 70°C for 20 minutes Standard Reaction Time: 1 hour	

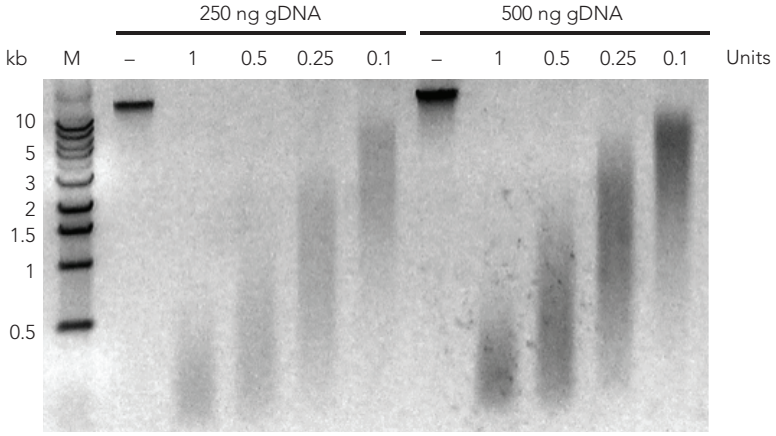
dsDNA Shearase™ Plus

Highlights:

- **Simple:** The simplest method for generating random-end dsDNA fragments.
- **Tunable:** Fragment size is easily controlled by adjusting enzyme concentration.
- **NGS-Ready:** dsDNA Shearase™ Plus-generated fragments are ideal for library construction, NGS, and DNA immunoprecipitation (i.e. MeDIP, MeDIP-Seq).

Description:

Digestion with dsDNA Shearase™ Plus is the simplest method for DNA fragmentation, as it circumvents the use of otherwise costly and cumbersome mechanical shearing devices. dsDNA Shearase™ Plus is an endonuclease that cleaves phosphodiester bonds in DNA to yield oligonucleotides with 5′-phosphate and 3′-hydroxyl termini. It has a particularly strong preference for double-stranded DNA (dsDNA) and generates random-ended DNA fragments of the desired size in a single-step. Sequencing data demonstrates that this enzyme does not introduce any detectable bias in the sequencing library preparation. It is compatible with low-volume inputs, thus minimizing sample loss. Digested DNA is easily purified in ≥ 6 µl with the recommended DNA Clean & Concentrator® technology (page 79) making it ideal for use in end modification (linker & adapter) procedures and other applications.



DNA is effectively fragmented using dsDNA Shearase™ Plus. 250 ng or 500 ng of HCT116 cell genomic DNA was incubated with 1, 0.5, 0.25, or 0.1 U dsDNA Shearase™ Plus for 20 min at 42°C. The reaction was stopped by incubating at 65°C for 5 min. Fragmented DNA was purified using the DNA Clean & Concentrator® kit and subsequently resolved in a 1% agarose gel. The amount of DNA fragmentation observed was directly correlated to the amount of enzyme used.

Product	Cat. No.	Size	Specification	Uses
dsDNA Shearase™ Plus	E2018-50 E2018-200	50 U 200 U	Enzyme Concentration: 1 U/µl Storage: -20°C Inactivation: 65°C for 5 minutes Standard Reaction Time: 20 minutes One unit (1 U) is defined at the amount of enzyme required to convert 250 ng human DNA into DNA fragments in the range of 100-500 bp in 20 minutes at 42°C in total reaction volume in 10 µl.	DNA fragmentation
dsDNA Shearase™ Plus with DNA Clean & Concentrator®-5	E2019-50 E2019-200	50 U+50 preps 200 U+200 preps