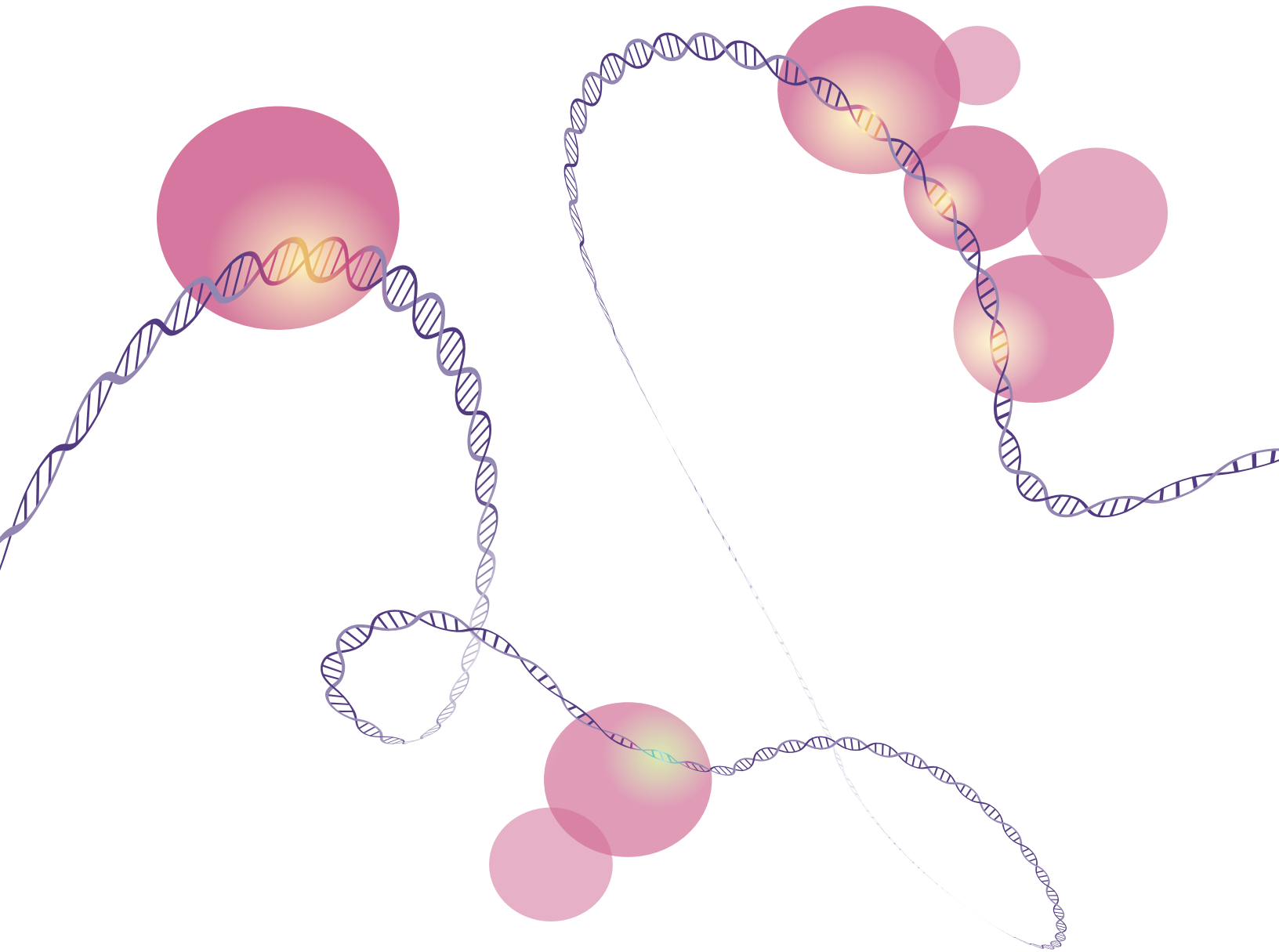


Products and Services to Study:

DNA Methylation



DNA methylation (5-mC) and methylation variants like 5-hmC contribute to mechanisms responsible for regulating gene expression and many other biological processes. These epigenetic modifications have also become important biomarkers for cancer, aging, neurological disorders, and many other human diseases.

Active Motif is proud to be the leading provider of products and services to study DNA methylation. Our comprehensive product line can support you whether you need just antibodies or recombinant proteins to do assays yourself or end-to-end epigenetic services in which our experts perform the assays, NGS, and bioinformatics for you.



Methylated DNA Enrichment Methods

Methylated DNA Immunoprecipitation (MeDIP) and Hydroxymethylated DNA Immunoprecipitation (hMeDIP) are techniques in which an antibody specific for either 5-mC or 5-hmC is used to selectively tag methylated genomic DNA fragments. Protein G magnetic beads are used to capture the antibody tagged fragments. MethylCollector™ Ultra is based on the Methylated CpG Island Recovery Assay (MIRA) which utilizes a His-tagged recombinant methyl-binding protein complex, MBD2b/MBD3L1, that specifically binds methylated CpGs in genomic DNA fragments. Nickel-coated magnetic beads capture the protein-DNA complexes. Enriched methylated DNA fragments can either be amplified via qPCR or sequenced.

Products & Services for Genome-Wide & Gene-Specific Analysis

Product/Service	Format	Catalog Number
MeDIP Kit	10 rxns	55009
hMeDIP Kit	10 rxns	55010
MethylPath™ MeDIP-Seq Service	Custom-Contact Us	25025
MethylPath™ hMeDIP-Seq Service	Custom-Contact Us	25052
MethylCollector™ Ultra Kit	30 rxns	55005

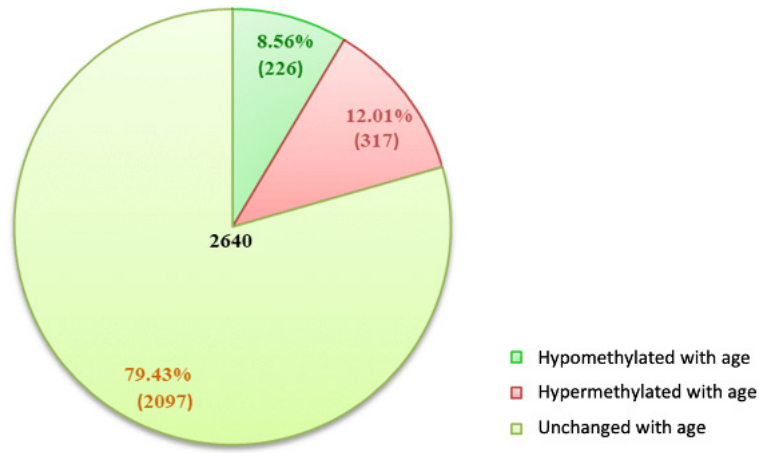


Figure 1: MeDIP-Seq Uncovers Aging-associated Methylation Patterns in Cell-Free DNA (cfDNA).

Serum samples were collected in serum separator tubes (SST) from healthy “normal” adults, two <25 yo and two >60 yo. Cell-Free DNA (cfDNA) was purified with the [Active Motif Cell-Free DNA Purification Kit \(Cat no. 25503/25504\)](#) and quantified with a Qubit fluorometer. 200 ng cfDNA was used in the MeDIP Kit and libraries were prepared using the [Swift Biosciences Accel-NGS™ 1S PLUS DNA Library Kit \(Cat no. 10024\)](#). Input to library prep was determined using AluI quantification. MeDIP and Input libraries were sequenced (38bp PE chemistry on Next-Seq500) and processed as per Active Motif’s standard pipeline for MeDIP-Seq. Peaks called using MACS2 relative to input (p -value cutoff = 1.00×10^{-7}).

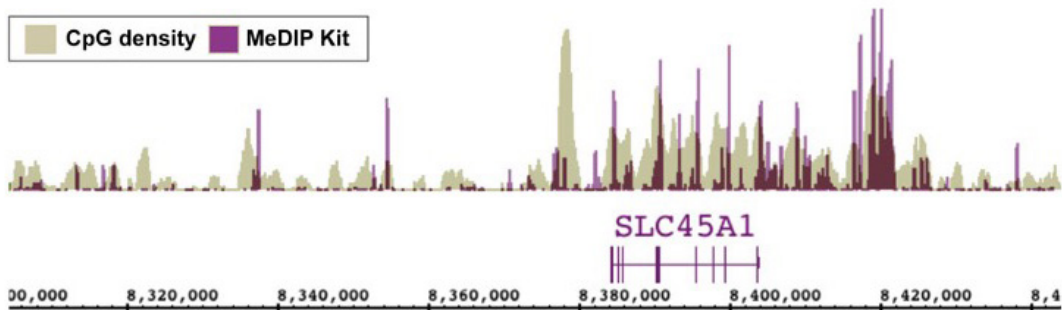
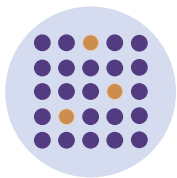


Figure 2: NGS Data generated from MeDIP enriched DNA fragments from PBMCs.

DNA was enriched from 1 µg of adaptor-ligated human PBMC DNA using Active Motif’s MeDIP Kit. NGS was performed using the Illumina platform to generate 26 million sequence tags. Tags were mapped to generate a whole-genome DNA methylation profile. The image above shows that the enriched regions (purple peaks) correlate well with CpG density (tan peaks).



Global Analysis of DNA Methylation

Global ELISA-based DNA methylation assays are used to investigate the overall levels of 5-mC or 5-hmC in samples to determine whether treatment conditions or disease phenotypes change epigenetic profiles. Clinical researchers use these high-throughput assays when screening large numbers of patient samples from their study cohorts.

Products Available to Study Global DNA Methylation Levels

Product	Format	Catalog Number
Global DNA Methylation Assay-LINE-1	1 x 96 rxns	55017
Global 5-hmC Quantification Kit	1 x 96 rxns	55025

LINE-1 Methylation in cfDNA from Human Serum

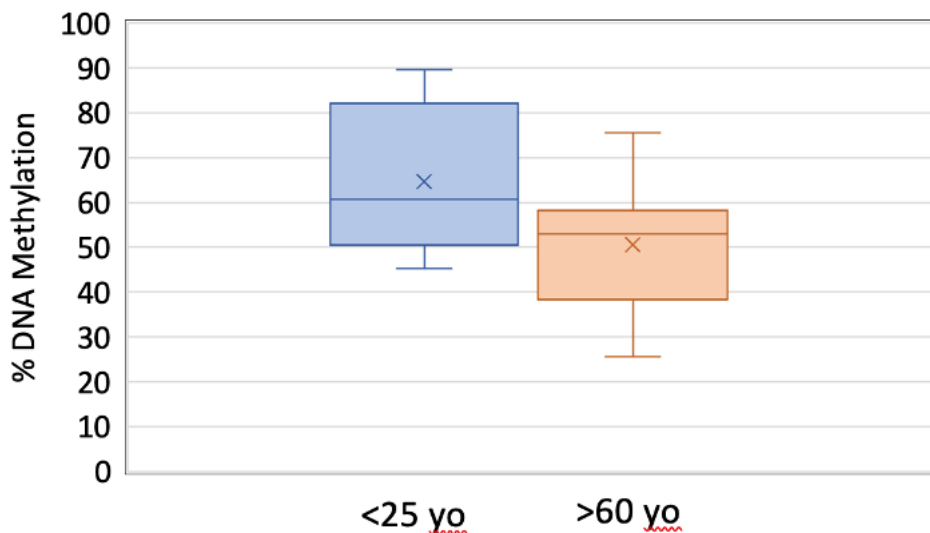


Figure 3: Global DNA Methylation – LINE-1 Assay shows changes in 5-mC levels in cfDNA with age.

Human serum samples from healthy “normal” adults <25 yo (N=6, mean: 21.83) and >60 yo (N=9, mean:65.89) were collected in serum separator tubes (SST). cfDNA was extracted using Active Motif’s Cell-Free DNA (cfDNA) Purification Kit (Cat No. [25503/25504](#)) from 5ml of serum and quantified using a Qubit fluorometer. Following the Global DNA Methylation Line-1 Kit protocol, without MseI digestion, 25 ng of cfDNA per well was used. The % 5-mC for each sample was calculated as a percentage of detectable CpG residues in the LINE-1 probe region, using the methylated DNA standards included in the kit. All samples were done in triplicate. Boxplot shows 25%-75% (), min-max (|), average (x), and median (-) % 5-mC.



Single-Nucleotide Resolution Analysis of DNA Methylation

When researchers need to know precisely which cytosine residues are methylated, as well as the overall levels of methylation, at the region(s) of interest, a DNA methylation assay with single-nucleotide resolution is required. The gold standard methods for investigating DNA methylation at single-nucleotide resolution involve performing bisulfite conversion.

Products & Services for Single-Nucleotide Resolution 5-mC Analysis

Product/Service	Format	Catalog Number
Reduced Representation Bisulfite Sequencing (RRBS)	Custom-Contact Us	25069
Targeted Bisulfite Sequencing Service	Custom-Contact Us	25035
FFPE Bisulfite Conversion Kit	40 rxns	55021
Bisulfite Conversion Kit	50 rxns	55016

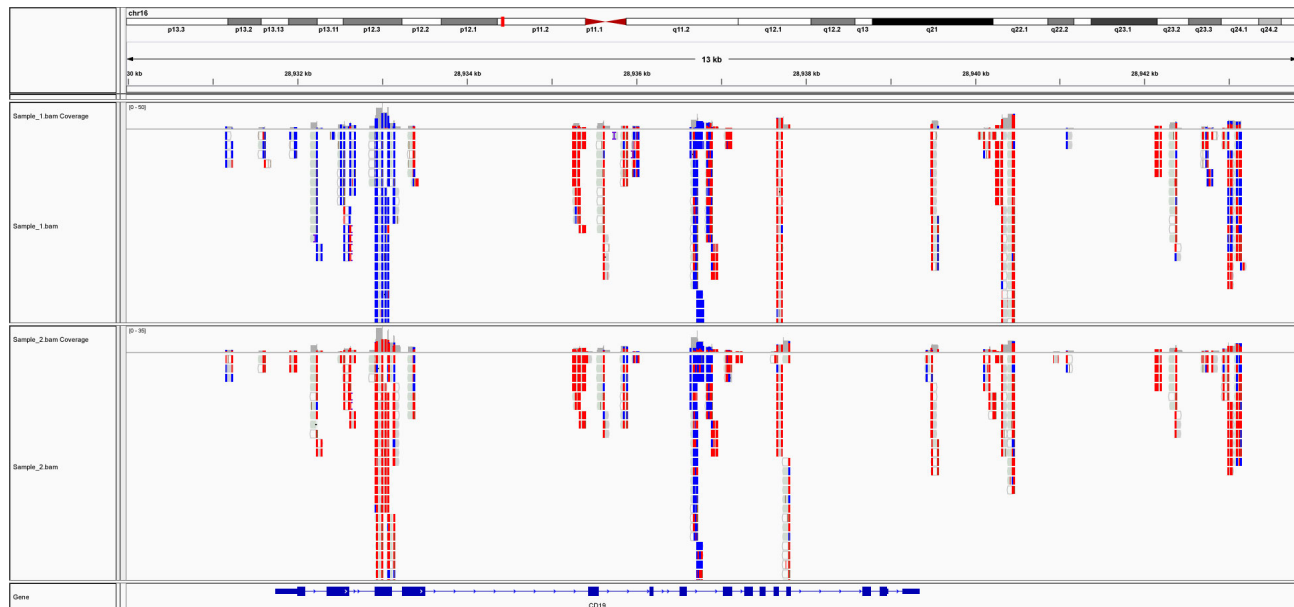


Figure 4: Differential Methylation in RRBS Samples.

The displayed regions are representative regions from the genome-wide data set and shows differential DNA methylation at an exon of CD19. Each block is a separate data point with red representing a methylated cytosine and blue representing an unmethylated base.



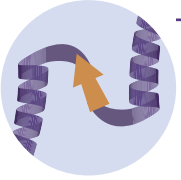
DNA Methylation Antibodies

The best DNA methylation assays start with the best antibodies, and Active Motif offers the highest quality and most complete line of antibodies available to study DNA methylation.

Antibodies to Study DNA Methylation

Featured Antibodies	Applications	Formats	Catalog Number
AbFlex® 5-methylcytosine antibody (rAb)	DB	100 µg	91187
5-Methylcytosine (5-mC) antibody (mAb)	DB, MeDIP, MeDIP-Seq, IF	100 µg	61479
5-Methylcytosine (5-mC) antibody (mAb)	MeDIP, IHC, ELISA	50 µg	39649
5-Hydroxymethylcytosine (5-hmC) antibody (mAb)	DB, MeDIP, ChIP-qPCR, ICC/IF, IHC(P)	100 µg	39999
5-Hydroxymethylcytosine (5-hmC) antibody (pAb)	DB, MeDIP, MeDIP-Seq, ICC/IF, IHC(P)	100 µg	39791

For a complete list of antibodies
visit [activemotif.com/dnamethabs](https://www.activemotif.com/dnamethabs)



DNA Methylation Enzymes

High-quality recombinant enzymes help improve development and optimization of biochemical assays, including high-throughput drug discovery screens.

Recombinant Enzymes

Enzyme	Format	Catalog Number
Recombinant TET1 (1418-2136) protein	20 µg	31417
Recombinant DNMT1 protein	20 µg/1 mg	31404/31800
Recombinant DNMT3A protein	20 µg/1 mg	31406/31802
Recombinant DNMT3A (278-432) protein	100 µg/1 mg	31541/31941
Recombinant DNMT3A / DNMT3L complex	20 µg/1 mg	31415/31815
Recombinant DNMT3B protein	20 µg/1 mg	31413/31813
Recombinant DNMT3B (212-358) protein	100 µg/1 mg	31542/31942
Recombinant DNMT3B / DNMT3L complex	20 µg/1 mg	31416/31816
Recombinant DNMT3L protein	20 µg/1 mg	31414/31814

For a complete list of DNA Methylation Proteins & Enzymes
visit [activemotif.com/epiproteins](https://www.activemotif.com/epiproteins)



DNA Methylation Assays

DNA methyltransferase (DNMT) enzymes are dysregulated in many cancers and other human diseases, making them attractive drug development targets. This assay allows studying DNMT activity in a high-throughput manner.

DNA Methylation Activity Assay

Product	Format	Catalog Number
DNMT Activity/Inhibition Assay	1 x 96 rxns	55006

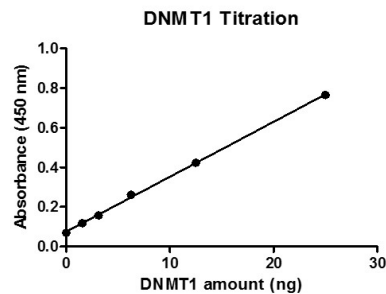


Fig. 5: Recombinant DNMT1 protein activity tested using DNMT Activity/Inhibition Assay.

Assay was performed using DNMT Activity / Inhibition Assay, Active Motif Catalog No. [55006](#). The substrate, AdoMet and DNMT1 were incubated for 1 hour at 37°C. The whole reactions were developed for 4 min.



DNA Methylation Accessory Products and Controls

Successful DNA methylation assays that can be reliably interpreted require using high-quality sample preparation methods and robust controls you can trust.

Sample Preparation & Control Products

Enzyme	Format	Catalog Number
Cell-Free DNA (cfDNA) Purification Kit	100 ml/250 ml	25503/25504
Methylated DNA Standard Kit	3 x 2.5 µg	55008
Fully Methylated Jurkat DNA	10 µg	55003



The Cell-Free DNA (cfDNA) Purification Kit is fully compatible with the KingFisher Flex and Duo. Please contact Active Motive technical support for the script files.



Publications

For a complete list of Active Motif Reagents and Services published for DNA Methylation Research, visit activemotif.com/publications.

Additional Resources

Learn more by visiting our [Epigenetics Resources](#) center – a complete series of Webinars, eBooks, and TechNotes designed to help you learn more about these methods and to stay up-to-date!

