ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

GETTING STARTED WITH GENOME-WIDE 5-mC & 5-hmC SEQUENCING

It is now well established that the classic genetic code is not sufficient to explain many complex phenotypes and causes of human diseases. Epigenetic modifications to DNA, such as methylcytosine (5-mC) and hydroxymethylcytosine (5-hmC), must also be considered as they can have profound effects on gene expression and are important for nearly every aspect of biology. These modifications can be stable, inheritable, and passed on to future generations.

With recent advances in sample preparation, high-throughput sequencing, and bioinformatics, researchers looking to study DNA methylation and hydroxymethylation now have several options for investigating these epigenetic modifications on a genome-wide scale. Zymo Research offers a comprehensive suite of epigenetic services, including multiple platforms for 5-mC and 5-hmC analysis for focused, mid-tier, and whole-genome interrogations.

The following technologies offer varying levels of resolving power, with some offering regional estimates of DNA methylation status, while others provide quantitative values at single-nucleotide resolution. Options are a good thing, but they can be a bit confusing at first. Here, we give a brief overview of the services we offer for DNA methylation and hydroxymethylation analysis, and some insights as to what approach might be best suited to your needs.

In this guide we will:

- Review the latest technologies for genome-wide 5-mC and 5-hmC analysis
- Recommend an experimental approach that suits your needs and budget

5-mC Analysis

- Reduced Representation Bisulfite Sequencing (RRBS)
- Whole-Genome Bisulfite Sequencing (WGBS)
- Targeted 5-mC Sequencing

5-hmC Analysis

- Genome-Wide 5-hmC CapSeq™
- Reduced Representation Hydroxymethylation Profiling (RRHP™)

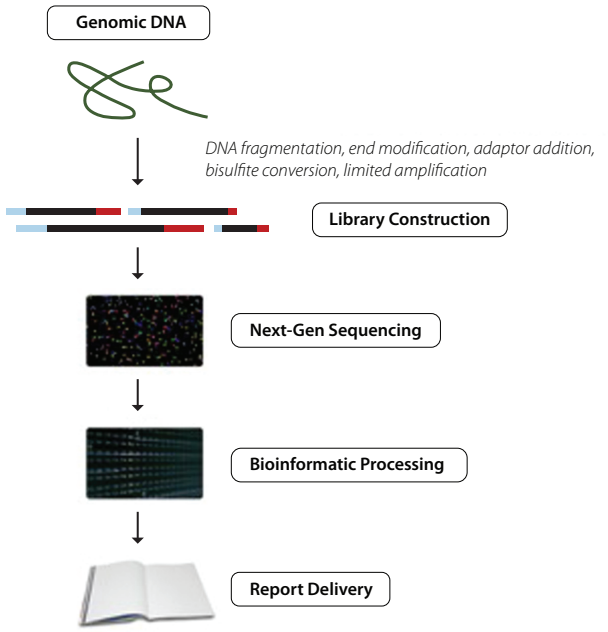
REDUCED REPRESENTATION BISULFITE SEQUENCING (RRBS)

Originally developed by Meissner and colleagues, reduced representation bisulfite sequencing (RRBS) is a powerful method that allows bisulfite sequencing at CpG-rich regions where DNA methylation most frequently occurs (1). This approach features restriction enzymes that digest CpG motifs, and digested samples are then prepped for high-throughput sequencing and bisulfite converted. This approach is beneficial for two reasons: 1.) It reduces sequencing costs substantially relative to whole-genome bisulfite sequencing while increasing read depth at CpG-rich regions, and 2.) It provides single-nucleotide resolving power with a quantitative readout of methylation frequency.

Zymo Research offers two variations of RRBS-derived approaches that have been further optimized to provide better genomic coverage and improved sequencing depth:

- Methyl-MiniSeq™ is based on the proven RRBS method, but is enhanced with a novel library prep workflow to increase both genome coverage and sequencing depth. Methyl-MiniSeq™ covers 2-3 million CpG sites in the human genome.
- Methyl-MidiSeq™ employs a similar methodology to Methyl-MiniSeq™, but includes additional restriction enzymes, so the coverage is expanded to include to 7-9 million CpGs in the human genome.

DNA Methylation Sequencing Workflow



WHOLE-GENOME BISULFITE SEQUENCING (WGBS)

Whole-Genome Bisulfite Sequencing, or WGBS, is a process that profiles DNA methylation across the entire genome. WGBS is the only approach that provides a complete picture of the entire DNA methylome at single-nucleotide resolution. These benefits come with increased sequencing costs, however, due to larger amount sequencing required for a high level of genomic coverage.

For researchers requiring this level of DNA methylation analysis, we offer:

- Methyl-MaxiSeq™ which is a validated and refined method for whole-genome bisulfite sequencing that provides maximum coverage of the DNA methylome, independent of CpG density.

GENOME-WIDE 5-hmC CAPSEQ™

Standard bisulfite-based sequencing approaches are powerful methods to interrogate DNA methylation, but they cannot distinguish between 5-mC and 5-hmC. For researchers looking to better understand 5-hmC in their models of study, Genome-Wide 5-hmC Capture Sequencing (CapSeq) is a very useful approach.

In this method, DNA is fragmented and hydroxymethylated cytosines are tagged with glucose moieties to create glucosylated 5-hmC (g-5-hmC). All hydroxymethylated DNA can then be enriched using J-binding protein 1 (JBP1), which has a strong affinity and extremely high specificity for g-5-hmC. This approach allows for mapping of 5-hmC on a genome-wide scale with much lower background noise contributed by 5-mC and/or unmodified cytosines, relative to antibody-based enrichment strategies.

Genome-Wide 5-hmC CapSeq™ is useful for localizing and determining the prevalence of 5-hmC as 'peaks' that indicate regions of high 5-hmC content across the genome. For many investigators, this level of resolution is ideal for identifying hydroxymethylated genomic regulatory elements and for drawing robust comparisons between samples.

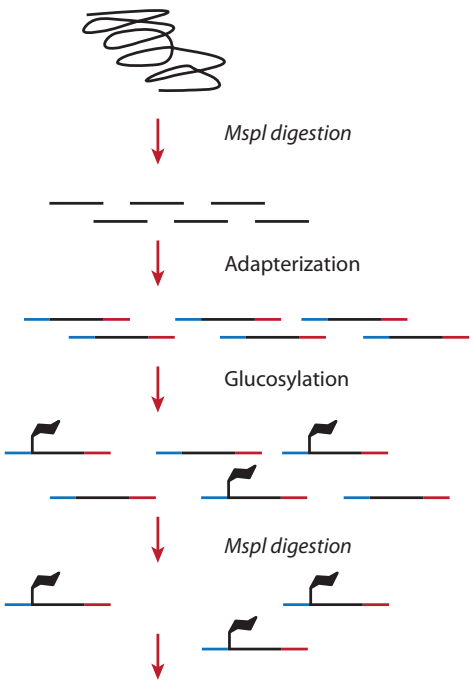
REDUCED REPRESENTATION HYDROXYMETHYLATION PROFILING (RRHP™)

Scientists at Zymo Research have recently developed a new approach called Reduced Representation Hydroxymethylation Profiling to enable single-nucleotide 5-hmC profiling on a genome-wide basis. In RRHP™, DNA is specifically fragmented and then size selected to focus the analysis on areas with the most hydroxymethylation information. Then, following library prep, 5-hmC is glucosylated and the library is digested once more with restriction enzymes sensitive to glucosylation. This approach not only allows for single-nucleotide 5-hmC profiling, but also provides quantitative and strand-specific information about 5-hmC distribution, as well as simultaneous detection of SNP positions within reads. RRHP™ is the first commercially available service for single-nucleotide resolution, genome-wide profiling of 5-hmC.

CONSIDERATIONS FOR DNA METHYLATION & HYDROXYMETHYLATION ANALYSIS STUDIES

When working with new clients, we first discuss their experimental needs, and then explain the available service options. Our intention is to provide the best possible option for our clients based on their needs, while still fitting realistically within their budget. High-throughput sequencing has become much less costly in recent years, and now, the services offered by Zymo Research make genome-wide epigenetic analyses available to all researchers. The services are customizable and 5-mC and 5-hmC analyses can be combined to get the most complete picture of your research. Furthermore, we do all of the bioinformatics analysis, data handling, and processing, making the results easy to interpret. All one needs to get started is an interest in epigenetic analysis. What follows are some of the recommendations we make to researchers interested in genome-wide DNA methylation and hydroxymethylation analysis:

Reduced Representation Hydroxymethylation Profiling Workflow



Schematic overview of the Reduced Representation Hydroxymethylation Profiling (RRHP™) system. The assay exploits β-glucosyltransferase (β-GT) to selectively label 5-hmC positions at adapter junctions, thus preventing digestion of the adapter away from the fragment. Fragments lacking 5-hmC at the junction will not be labeled and the adapter can be digested away. Only fragments with intact adapters on both sides will be amplified for hybridization and sequencing.

5-mC SEQUENCING ON A BUDGET

For researchers on a limited budget, but who still want learn more about DNA methylation in their model system, we recommend our Methyl-MiniSeq™. It provides good coverage of nearly all genetic regulatory regions, promoters, and CpG islands without being too costly. Methyl-MiniSeq™ is also a great option for biomarker discovery as it covers the regions of the genome with highest levels of DNA methylation (~2-3 million CpGs in the human genome).

5-mC SEQUENCING FOR NEWBIES

Methyl-MidiSeq™ is a great starting point for researchers who are just getting acquainted with the field and may not know how important DNA methylation is in their model system. It provides enough information to gain a solid understanding of DNA methylation without breaking the bank. Methyl-MidiSeq™ profiles significantly more positions (~7-9 million CpGs in the human genome) relative to Methyl-MiniSeq™, which is important to make sure you don't miss anything when you are not yet sure where to look.

THE WHOLE ENCHILADA - BROAD COVERAGE 5-mC SEQUENCING

When it is coverage you need, there is no substitute for Zymo's Methyl-MaxiSeq™. This WGBS-based technology will deliver more DNA methylation information than any other approach. If you have already determined DNA methylation is key in your research, and you would like to analyze it across the entire genome, then this is the method for you.

THE BEST APPROACH FOR TARGETED DNA METHYLATION SEQUENCING

Many researchers have already identified key genes, differentially methylated regions, or regulatory regions of interest that are relevant in their field of study and wish to profile larger numbers of samples at these regions versus needing increased genome-wide coverage.

So, we recommend a targeted DNA methylation sequencing approach if you're interested in dozens, or even a few hundred regions. For this, we offer a proprietary multiplexing strategy to analyze multiple targeted regions in a single reaction that allows high-throughput sequencing and keeps costs low. Sequenced targets will routinely have >1,000-fold coverage. This allows quantitative analysis of even extremely low DNA methylation levels.

GENOME-WIDE 5-hmC SEQUENCING

For labs interested in identifying genomic regions that are hydroxymethylated, Genome-Wide 5-hmC CapSeq™ is a great method, as it will provide the researcher a snapshot of 5-hmC across the entire genome. This method has the benefit of interrogating 5-hmC across all genomic regions, without any sequence requirements or dependence on restriction site availability, and is ideal when larger amounts of DNA (i.e. microgram or higher) are available.

QUANTITATIVE SINGLE-NUCLEOTIDE RESOLUTION 5-hmC PROFILING

If you are interested in profiling 5-hmC modifications at single-nucleotide resolution, then RRHP™ is the only method that is ideal. RRHP™ also has the added benefits of: 1) being compatible with small inputs of DNA (~100 ng), 2) being quantitative and, 3) having the ability to pin 5-hmC to a precise base pair and to a specific DNA strand. Finally, due to similar fragmentation strategies, RRHP™ results can be overlaid with Methyl-MiniSeq™ data for simultaneous 5-mC and 5-hmC profiling.

DNA METHYLATION & HYDROXYMETHYLATION SEQUENCING: KEY TAKEAWAYS

The epigenetic services offered by Zymo Research take the guesswork and uncertainty out of genome-wide analysis of DNA methylation and hydroxymethylation. Zymo has over a decade of experience in providing tools to epigenetic researchers, and our chemistries for bisulfite conversion remain the most cited and most trusted methods available. Our services allow anyone who is interested in investigating how epigenetic modifications play a role in their research to do so, on a genome-wide scale, without the requirements of needing their own Next-Gen sequencing equipment or a bioinformatics department. Zymo provides complete and customizable bioinformatics solutions and all services can be performed on samples from any organism for which there is a reference genome. Simply provide us with the samples and we will return the results as a consolidated easy-to-interpret report with publication-ready graphs and tables. If you have any questions about what the best approach for you is, please contact our knowledgeable service team at: services@zymoresearch.com and discuss your project today.

SERVICES FOR GENOME-WIDE DNA METHYLATION & HYDROXYMETHYLATION ANALYSIS

Epigenetic Sequencing Approach				
DNA Modification	Method	Coverage (in human genome)	Resolution	Price
5-mC	Methyl-MiniSeq™	3-4 Million UniqueSites	Single Nucleotide	$
	Methyl-MidiSeq™	8-9 Million Unique Sites	Single Nucleotide	$$
	Methyl-MaxiSeq™	Complete Genome	Single Nucleotide	$$$
	Targeted Bisulfite Sequencing	A Few to Hundreds of Sites	Single Nucleotide	$-$$
5-hmC	Reduced Representation Hydroxymethylation Profiling (RRHP™)	2-3 Million Unique Sites	Single Nucleotide	$
	Genome-Wide 5-hmC CapSeq™	Nearly all Unique 5-hmC Sites	Peaks	$

References:

1. Methods. 2009 July; 48(3): 226–232

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