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DNA Methylation Analysis

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Abstract

Methylation at CpG dinucleotides is a chemical modification of DNA hypothesized to play important roles in regulating gene expression. In particular, the methylation of clusters of methylation sites, called “CpG islands”, near promoters and other gene regulatory elements may contribute to the stable silencing of genes, for example, during epigenetic processes such as genomic imprinting and X-chromosome inactivation. At the same time, aberrant CpG methylation has been shown to be associated with cancer.

In this video, the biological functions and mechanisms of DNA methylation will be presented, along with various techniques used to identify methylation sites in the genome. We will then examine the steps of bisulfite analysis, one of the most commonly used methods for detecting DNA methylation, as well as several applications of this technique.

Transcript

DNA methylation is a chemical modification of DNA that affects gene expression under different cellular contexts. Many researchers are interested in the mechanism and functions of this process, as aberrant DNA methylation has been associated with diseases such as cancer.

In this video, we will cover the principles behind DNA methylation and methods to detect it. Then, we will explore a general protocol for one of these methods, bisulfite analysis, and some applications of this technique.

First, let's take a look at what DNA methylation is and how it can be detected.

During this biochemical process, a cell adds a chemical tag known as a methyl group to cytosine bases in its DNA. Most methylated cytosines occur next to guanine bases in the same DNA strand, and these adjacent nucleotides—and the phosphodiester bond that links them—are referred to as “CpGs.” Although most vertebrate CpGs are methylated, those that are unmethylated tend to occur close together in CpG “islands” near the promoters of actively expressed genes, and various other regulatory sequence elements.

How changes in DNA methylation contribute to gene regulation is still a subject of intense investigation. Methylation of CpG islands seems to be important for the stable, long-term gene silencing seen in epigenetic processes such as genomic imprinting, which is the parent-of-origin specific expression of certain genes, as well as X-chromosome inactivation, the silencing of one of the two X-chromosomes in each cell of female mammals. Repression of critical genes due to aberrant methylation of CpG islands has also been shown to contribute to uncontrolled cell growth, which can lead to cancer. Mechanistically, DNA methylation may contribute to gene silencing by either preventing transcription factors from associating with promoters, or by recruiting proteins that modify histones and remodel chromatin into a transcriptionally non-permissive state.

There are several methods for detecting the methylation state of DNA.

One technique, the HELP assay, involves two restriction endonucleases called *HpaII* and *MspI*, which respectively cleave only unmethylated, or both methylated and unmethylated, CCGG sequences. By comparing the digestion patterns produced by these two enzymes, the methylation status of DNA can be deduced.

Another method, called methylated DNA immunoprecipitation or “MeDIP,” uses antibodies that bind to methylated cytosines to enrich for methylated DNA sequences.

Finally, bisulfite analysis is used to distinguish methylated from unmethylated cytosine in DNA, by carrying out a chemical reaction that converts unmethylated cytosine to uracil. Following this conversion, bisulfite-treated DNA can be subjected to PCR, sequenced, and compared to a reference genome. Unmethylated cytosines are those that are present in the reference, but replaced with thymines following bisulfite analysis and PCR. By subjecting bisulfite-treated DNA to mass spectrometry, researchers can also create “methylation epigrams,” which linearly represent different CpGs in the genome and depict the degree of methylation at each of them. Such epigrams are particularly useful if researchers wish to compare methylation patterns between different cell types.

Let's now take an in-depth look at the protocol for bisulfite analysis.

To begin, sodium hydroxide is added to preparations of genomic DNA, which are then incubated at 95°C. This denatures the DNA and makes its bases accessible to subsequent chemical reactions. Sodium metabisulfite is then introduced into the denatured DNA mixture, and two chemical reaction steps will occur. During the first step, sulfonation, a sulfite group is added to an unmethylated cytosine to form cytosine sulfonate. Then, during hydrolytic deamination, an amino group is removed from cytosine sulfonate to generate uracil sulfonate.

To facilitate these first stages of the chemical reaction, the DNA preparation is overlain with mineral oil, which prevents evaporation and helps to maintain the concentration of sodium metabisulfite. The reaction is then incubated at 55°C in the dark. During this step, an agent to prevent oxidation, such as quinol, is also added to the mixture.

To collect modified DNA containing uracil sulfonate, and no mineral oil, the mixture is centrifuged and the lowest liquid layer recovered. The DNA in this solution is then purified.

Next, sodium hydroxide is added to the DNA mixture, which is then incubated at 37°C. This is done to induce desulfonation, which—as you may have guessed—removes the sulfite group from uracil sulfonate, forming uracil and completing the chemical reaction.

Finally, the mixture is neutralized with the addition of ammonium acetate, and the DNA is collected by ethanol precipitation. Once the bisulfite-converted DNA has been purified, it is subjected to PCR and sequencing.

Now that we have discussed the basic technique for bisulfite analysis, let's look at some experimental applications.

Some researchers use bisulfite analysis to investigate genomic imprinting. Here, researchers crossed two strains of *Arabidopsis* with genetic differences, so that maternal and paternal DNA could be distinguished. Methylation patterns in the resulting embryos and associated endosperm, or the tissue that supports the embryo, were then compared. Using this method, scientists found that CpGs in the maternal allele of a protein-encoding gene, *MEA*, tended to be methylated in embryos but unmethylated in endosperm, indicating tissue-specific imprinting.

Other researchers are using this technique to understand how environmental or social factors can alter methylation patterns. Here, mouse pups were separated from their mothers to induce stress, and their brain tissues were subsequently isolated. Following sequencing of bisulfite-treated DNA, scientists determined that methylation patterns in a hormone-encoding gene, *AVP*, changed in a specific brain region in “separated” pups, suggesting a possible molecular mechanism for long-term biological effects of early life experience.

Finally, many researchers are trying to optimize bisulfite analysis to facilitate the comparison of methylation patterns between individual, unique cells. Here, researchers modified the bisulfite analysis method so that all steps were performed on individual mouse oocytes embedded in agarose, which helped to guard against DNA loss. Using this method, researchers were able to easily identify single-oocyte samples that had been contaminated with other cells by looking for those that gave multiple methylation patterns.

You've just watched JoVE's video on methylation sensitive analyses. Here, we've discussed the role that DNA methylation plays in gene regulation, methods that researchers use to identify methylated regions in the genome, a generalized protocol for bisulfite analysis, and finally, some applications of this technique. As always, thanks for watching!