Title: DNA Methylation Analysis

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.

Application videos:

1. Determination of DNA Methylation of Imprinted Genes in Arabidopsis Endosperm **(2327 Thumbnail @ 2:28 – Microscope view of flower getting pollinated)**

Description: Genomic imprinting is an epigenetic phenomenon where, for certain genes, only the copy inherited from a particular parent is ever expressed in the offspring, and DNA methylation has been found to play a role in this process. Here, researchers use bisulfite analysis followed by sequencing to compare DNA methylation patterns in *Arabidopsis* embryo and endosperm (the supporting tissue in the seed), which develop from separate fertilization events. Hybrid plants, established by crossing two different parental lines, are used here so that the maternal and paternal alleles can be readily distinguished.

2. Optimized Analysis of DNA Methylation and Gene Expression from Small, Anatomically-defined Areas of the Brain **(3938 Thumbnail @ 11:17 Mouse brain slice)**

Description: Evidence suggests that environmental factors such as early-life stress (ELS) may cause epigenetic alterations that last throughout an organism’s lifetime. In this article, scientists separated newborn mouse pups from their mother to induce ELS, then following tissue isolation from neuroanatomically defined brain regions, bisulfite sequencing was performed to analyze DNA methylation status at genomic loci of interest.

3. Single Oocyte Bisulfite Mutagenesis **(4046 Thumbnail @ 2:50 – Microscopic view of single oocyte)**

Description: This video demonstrates a method for studying DNA methylation in single cells, in this case oocytes. Bisulfite analysis of DNA isolated from individual cells eliminates issues of PCR bias that might arise when samples are pooled. This protocol also allows for easily identifying contaminated samples, as indicated by the detection of multiple methylation patterns in different reactions using the same starting materials.

4. Enhanced Reduced Representation Bisulfite Sequencing for Assessment of DNA Methylation at Base Pair Resolution **(52246 Thumbnail @11:54 Multiple results images)**

Description: Bisulfite analysis has been modified in a number of ways to make it more suitable for genome-scale analysis. One of these is reduced representation bisulfite sequencing (RRBS), which enriches for GC-rich regions of the genome, reducing the “depth” (average number of times each basepair in the library is sequenced), and thus the cost, needed to detect methylated CpG sequences. This protocol uses the methylation-insensitive restriction enzyme *MspI*, which cuts at CCGG sites, to generate small DNA fragments with GC sequences at their ends. These are then size-selected, bisulfite converted, and used to generate next-generation sequencing libraries, allowing for quantitative, single-basepair resolution of methylation status from small quantities of test material. A further modification that addresses some limitations in the original RRSB technique, called enhanced RRSB, is presented in this video.

5. DNA Methylation: Bisulphite Modification and Analysis **(3170 Thumbnail @ 11:57 – Results image of DNA methylation sites)**

Description: This article details the protocol of bisulfite analysis of DNA methylation. Following DNA isolation, the step-by-step reactions for bisulphite deamination, which results in unmethylated cytosines being converted to uracil residues, are presented. This is followed by PCR with primers specifically amplifying only sequences that have been bisulfite-converted, and finally the PCR products are cloned and sequenced to distinguish between converted and unconverted cytosines.

Related Videos

5043 – Regulating Temperature in the Lab: Applying Heat

5056 – PCR: The Polymerase Chain Reaction

5332 – Embryonic Stem Cell Culture and Differentiation

5549 – Introduction to Epigenetics