Title: 5549- Introduction to Epigenetics

Abstract: Since the early days of genetics research, scientists have noted certain heritable phenotypic differences that are not due to differences in the nucleotide sequence of DNA. Current evidence suggests that these “epigenetic” phenomena might be controlled by a number of mechanisms, including the modification of DNA cytosine bases with methyl groups, the addition of various chemical groups to histone proteins, and the recruitment of protein factors to specific DNA sites via interactions with non-protein-coding RNAs.

In this video, JoVE presents the history of important discoveries in epigenetics, such as X-chromosome inactivation (XCI), the phenomenon where an entire X-chromosome is silenced in the cells of female mammals. Key questions and methods in the field are reviewed, including techniques to identify DNA sequences associated with different epigenetic modifications. Finally, we discuss how researchers are currently using these techniques to better understand the epigenetic regulation of gene function.

Application Videos:

1. High Sensitivity 5-hydroxymethylcytosine Detection in Balb/C Brain Tissue (**2661** **Thumbnail@0:43- schematic of experiment**)

Description: DNA methylation, or the addition of a methyl group to a specific carbon atom in a cytosine base, has long been regarded as an important epigenetic modification. In the past decade, however, it has been found that methylcytosine may be further modified to hydroxymethylcytosine, and the potential function of this modification remains unclear. This video describes a protocol that uses restriction enzymes to distinguish between DNA with these different types of methylation.

2. Chromatin Isolation by RNA Purification (ChIRP) (**3912** **Thumbnail@0:38- illustration of protocol**)

Description: A major mechanism hypothesized for the function of long noncoding RNAs (lncRNAs), or functional transcripts that are not translated into proteins, is that they recruit gene regulatory proteins to specific sites in the genome. The authors of this article present a novel technique called “chromatin isolation by RNA purification” or ChIRP, where a lncRNA of interest is isolated using oligonucleotide probes. DNA associated with this RNA can then be isolated and analyzed to determine all of its target genomic regions.

3. Quick Fluorescent *In Situ* Hybridization Protocol for Xist RNA Combined with Immunofluorescence of Histone Modification in X-chromosome Inactivation (**52053 Thumbnail @** **7:04-Remove text overlay-talent placing coverslip on slide**)

Description: In this video, researchers used fluorescence microscopy techniques to evaluate the genomic locations of both Xist, a lncRNA known to “coat” the X-chromosome during XCI, and a specific histone modification associated with gene silencing, termed H3K27me3. By looking for their colocalization in differentiating mouse embryonic stem cells undergoing XCI, insight can be gained into the mechanistic roles of lncRNA and histone modifications during this epigenetic process.

4. Associated Chromosome Trap for Identifying Long-range DNA Interactions (**2621 Thumbnail@0:19- schematic of interacting DNA molecules and restriction digest sites**)

Description: In addition to chemical groups and lncRNAs, epigenetic regulation can also be mediated by interactions between different regions of DNA on the same, or a different, chromosome. Here, researchers present a novel technique to evaluate such “long-range” interactions, termed “Associated Chromosome Trap”, which they used to compare how DNA interactions between imprinted genes and gene regulatory sites differed between normal and cancer cells.

5. The ChroP Approach Combines ChIP and Mass Spectrometry to Dissect Locus-specific Proteomic Landscapes of Chromatin (**51220 Thumbnail@8:19- SDS-PAGE gel image and histones schematic**)

Description: The “chromatin proteome” refers to all of the proteins and histone modifications that either constitute, or associate with, a particular stretch of chromatin. This video demonstrates a protocol to characterize the proteome of a specific chromatin region of interest, in this case transcriptionally silent chromatin associated with the histone mark H3K9me3. This technique pairs chromatin immunoprecipitation with mass spectrometry—which, respectively, isolates chromatin regions using antibodies against specific proteins, and identifies protein fragments based on their mass.

Related SciEd Videos:

5070 – Restriction Enzyme Digests

5058 – Separating Protein with SDS-PAGE

5328 – An Introduction to Molecular Developmental Biology

5550 – DNA Methylation Analyses