Title: 5551-Chromatin Immunoprecipitation

Abstract: Histones are proteins that help organize DNA in eukaryotic nuclei by serving as “scaffolds” around which DNA can be wrapped, forming a complex called “chromatin”. These proteins can be modified through the addition of chemical groups, and these changes affect gene expression. Researchers use a technique called chromatin immunoprecipitation (ChIP) to better understand which DNA regions associate with specific histone modifications or other gene regulatory proteins. Antibodies are used to isolate the protein of interest, and the bound DNA is extracted for analysis.

Here, JoVE presents the principles behind ChIP, discussing specific histone modifications and their relationship to gene expression and DNA organization. We then review how to perform a ChIP protocol, and explore the ways scientists are currently using this technique.

Application Videos:

1. Chromatin Immunoprecipitation (ChIP) to Assay Dynamic Histone Modification in Activated Gene Expression in Human Cells (**2053 Thumbnail@2:29- talent adding formaldehyde to stack of culture dishes)**

Description: Often, ChIP is used to isolate DNA associated with a particular histone modification, and is then paired with techniques such as real time PCR (also known as quantitative or qPCR) to accurately determine the amount of DNA from specific loci. In this article, researchers treated human cancer cell lines with a signaling molecule, and then used ChIP followed by qPCR to detect how the extent of histone modifications in a gene of interest changed in response to the length of treatment.

2. Chromatin Immunoprecipitation from Dorsal Root Ganglia Tissue Following Axonal Injury (**2803** **Thumbnail@7:09-Talent holding magnetic rack**)

Description: Other than histone modifications, ChIP can also be used to identify DNA associated with specific proteins. This video demonstrates how ChIP can help investigate the regulatory proteins that bind to DNA in response to tissue injury, potentially activating genes involved in regeneration.

3. Automating ChIP-seq Experiments to Generate Epigenetic Profiles on 10,000 HeLa Cells (**52150 Thumbnail@3:25-machine working with magnetic beads in plate**)

Description: Here, an “automated” ChIP protocol is presented, highlighting how a robotic system can be used to optimize this technique, and be further coupled to the automated construction of libraries for high-throughput sequencing. Researchers applied this method to generate genome-wide profiles for multiple histone modifications using a small sample of as little as 10,000 cells.

4. Chromatin Immunoprecipitation (ChIP) Using *Drosophila* Tissue (**3745 Thumbnail@12:28- graph showing ChIP profile**)

Description: Histone modifications can vary between different tissues, and may contribute to tissue-specific patterns of gene expression. In this video, testes were dissected from mutant flies with defects in sperm development, and their chromatin subjected to ChIP. Following analysis of the resulting DNA, researchers concluded that, in these mutants, the level of a specific histone modification associated with gene silencing—H3K27me3—was increased in three genes involved in sperm formation.

5. Detection of Histone Modifications in Plant Leaves (**3096 Thumbnail@5:21- talent pipetting solution away, with pellet of agarose beads visible at bottom of tube**)

Description: Pathogen infection in plants can result in the production of hormones, like salicylic acid, that regulate the expression of immune system genes. The authors of this article used ChIP to better understand the mechanisms behind pathogen-mediated gene expression in plants. They demonstrated that treatment of *Arabidopsis* with a synthetic hormone that mimicked infection resulted in an increase in histone acetylation at the promoter of a specific plant defense gene, *PR2*.

Related SciEd Videos:

5048 – Using a Hemocytometer to Count Cells

5093 – *Drosophila* Development and Reproduction

5339 – Tissue Regeneration with Somatic Stem Cells

5549 – Introduction to Epigenetics