

# METHODS IN MOLECULAR BIOLOGY™

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# Plant Epigenetics

## Methods and Protocols

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## Preface

The discovery of DNA as the genetic material brought great hope to scientists all over the world. It was believed that many of the lingering questions in genetics and the mechanisms of heredity would finally be answered. However, as often is the case in science, more questions arose out of this discovery. What defines a gene? What are the mechanisms of gene regulation? Further discovery and technological innovations brought about sequencing techniques that allowed the study of complete genomes from many organisms, including *Arabidopsis* and humans. Despite all the excitement surrounding these technologies, many features of the genome remained unclear. Peculiar characteristics in genome composition such as significant redundancy consisting of many repetitive elements and noncoding sequences, active transcriptional units with no protein product, and unusual sequences in promoter regions added to the mysteries of genetic make-up and gene regulation. Indeed, the more we discovered about the genome, the more difficult it became to understand the complexity of cellular function and regulation.

Out of the study of the intricacies of the genome and gene regulation, arose a new science that was independent of actual DNA changes, but critical in maintaining gene regulation and genetic stability. Epigenetics, literally translated as “above genetics,” is the science that describes the mechanisms of heritable changes in gene regulation that does not involve modifications of DNA sequence. These changes may last through somatic cell division and, in some cases, throughout multiple generations.

Epigenetics is perhaps one of the most popular and quickly evolving fields of modern science. Despite the fact that the ideas behind epigenetics had already been developing in the late nineteenth and early twentieth centuries, major advances have only occurred within the last 10–15 years as the mechanisms surrounding epigenetic regulation began to be uncovered. It was hoped by many that the mysteries of gene regulation and inheritance that remained unanswered would finally be elucidated with the help of this new science. Since, the understanding of the contribution of epigenetic regulation to cell function has helped scientists from many distinct fields of research such as molecular biology, population genetics, microbiology, ecology, developmental biology, and evolution.

Gene silencing as an epigenetic mechanism to control gene expression was first described in plants. This occurred with the beginning of the era of plant transgenesis, and almost undermined the new paradigm of improvement of plant performance via transgenic techniques. Silencing was a serendipitous discovery, as this finding revitalized the field of epigenetics. Phenomena such as plant acclimation and adaptation to stress, hybrid and heterozygote vigor (heterosis), plant tolerance to viral infection, transgenerational changes in genome stability, paramutations, among others, are now considered excellent candidates for regulation via epigenetic mechanisms. Future studies involving various protocols for the analysis of methylation patterns, histone modifications, chromatin structure, and small RNA expression, the hallmarks of epigenetic regulation, will undoubtedly help to explain these phenomena. It will be exciting to discover how plants utilize these mechanisms to adapt to stress, and how we can manipulate these characters for the generation of better and hardier crops.

In this book we have collected a variety of protocols for the study of the function of small noncoding RNAs, DNA methylation, and histone modifications in plants. Where possible and appropriate, we presented several protocols with different degrees of complexity. We also include protocols for plant transgenesis and the analysis of genome stability, with a discussion for their applications to epigenetic studies. It was our aim to put together a single manual that researchers in the field of plant epigenetics can turn to in hopes to answer the many yet undiscovered and unexplained phenomena in plant biology.

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