
Preface

The splicing of nuclear pre-mRNAs is carried out by the spliceosome, which recognizes splicing signals and catalyzes the removal of noncoding intronic sequences to assemble protein coding sequences into mature mRNA prior to export and translation. Of the approximately 25,000 genes encoded by the human genome, more than 90 % are believed to produce transcripts that are alternatively spliced. Thus, alternative splicing of pre-mRNAs can lead to the production of multiple protein isoforms from a single pre-mRNA, significantly enriching the proteomic diversity of higher eukaryotic organisms. Because regulation of this process can determine the timing and location that a particular protein isoform is produced, changes in alternative splicing patterns modulate many cellular activities. Consequently, the process of splicing must occur with a high degree of specificity and fidelity to ensure the appropriate expression of functional mRNAs.

Mutations in RNA splicing regulatory elements or in genes encoding splicing regulators that bind splicing regulatory elements can cause or modify the severity of disease. Early estimates, based on the identification of mutations within splice sites, suggested that ~15 % of all single base mutations change splicing patterns. However, it is now clear that many more mutations affect splicing by disrupting other important RNA elements, such as splicing enhancers or silencers binding sites. New estimates suggest that up to 60 % of known mutations could cause disease through changes in pre-mRNA splicing. A significant step towards identifying some of these disease-causing mutations has been made recently by combining novel high-throughput experimental and bioinformatic approaches to define splicing patterns and splicing regulatory elements. The advent of novel methods to analyze the activities of the spliceosome has led to the merging of different analytical disciplines. The goal of this book is to provide the reader with a guide to classical experimental approaches to decipher splicing mechanisms and to provide experimental strategies that rely on novel multidisciplinary approaches.

This book was written with graduate and medical students, clinicians, and postdoctoral researchers in mind. It describes the theory of alternative pre-mRNA splicing in seven introductory chapters and then introduces protocols and their theoretical background relevant for a variety of experimental research. These protocol chapters cover basic methods to detect splicing events, analyses of alternative pre-mRNA splicing *in vitro* and *in vivo*, manipulation of splicing events, and high-throughput and bioinformatic analyses of alternative splicing. Each chapter provides a theoretical introduction and a practical guide for molecular biologists, geneticists, clinicians, and every researcher interested in alternative splicing. In general, the protocols require a basic knowledge of molecular biology and/or RNA methods.

The protocols in this book are a collection of commonly used methods in the field of alternative splicing. These protocols should be viewed as guides for experiments that allow investigators to understand basic procedures. It is hoped that the chapters will allow readers

to quickly find the experimental tools necessary for their projects and that it will stimulate their interest in trying out other techniques. As such, I hope that this compendium of methods and protocols will help newcomers and seasoned molecular biologists to understand the fascinating world of alternative splicing with the ultimate goal of paving the way for many new discoveries to come.

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Methods and Protocols

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