
Preface

The scope of “Gene Correction: Methods and Protocols” is to provide a user-friendly, well-detailed, and up-to-date collection of many strategies and methodologies utilized for generating specific sequence changes in the DNA of cells in the laboratory and for tackling the major problems that the field of gene correction is facing. Now that DNA sequencing technology has become sensitive and reliable enough to enter routine clinical practice, it is easy to identify genetic defects in genomic DNA. Considering that there are thousands of genetic diseases that are caused by a single sequence defect in a gene, it is obvious that the best way to prevent or cure a genetic disease is by correcting the defective gene that is causing it. Thus, it is becoming more and more important to have the knowledge and the tools to edit DNA at will. As our skills to manipulate the genetic material of cells progressed dramatically in the last decade, we acquired novel techniques and remarkably enhanced our capacity to genetically engineer genes for the purpose of better understanding the molecular mechanisms of life, and also for directly fixing mutations that cause innumerable devastating and incurable diseases in humans. Nevertheless, editing the genetic information of DNA is a challenging task. The goal of gene correction goes far beyond the process of making a desired change in a chosen target gene in the most efficient way. It is essential that the product of the modified gene should then be functional, the DNA correction stable and the engineering process accurate and restrained to the target to minimize unwanted DNA, cellular, and/or tissue damage.

The strategies for gene modification are currently numerous and diverse and are subjected to continuous evolution, improvement, and optimization. This book brings together many experts in the field of gene correction to disclose a wide and varied array of specific gene correction protocols for engineering mutations in DNA, for delivering correcting DNA to target cells and for improving the accuracy and safety of the gene correction process. This book is aimed at an audience of scientists of all backgrounds interested in the area of gene targeting/recombination/therapy. The methodologies presented in this volume are carefully explained and detailed so that they can be easily learned and applied by researchers who are not initially familiar with the procedures. The objective is from scratch to success: starting with a comprehensive listing of the Materials, every chapter contains a step-by-step guidance through the Methods and a series of useful tips provided in the form of Notes intercalated into the text.

The book is informally divided into four sections based on topic. Because each chapter could belong in more than one section, at the end of each section I have added a list of those chapters that provide additional protocols for gene correction specific to the topic of that section. Thus, each section goes beyond the subject matter presented in the selected chapters, and better helps the reader to find the material of interest. Gene correction can be accomplished in many different organisms and cell types. The first section (Part I) presents a sample of gene correction approaches in hosts as different as *Pseudomonas*, *Drosophila*, chicken cells, and human pluripotent stem cells. Approaches for gene correction in these and many other different host organisms and cell types are presented throughout the book

in several other chapters; hence, these are reported at the end of the first section for useful reference. Similarly structured, the second section (Part II) centers on some of the most effective instruments for gene correction, comprising both nonviral and viral tools. The third section (Part III) contains protocols that emphasize the impact of inducing a break in the target DNA to stimulate gene correction, exploiting the positive features of break-induced gene targeting, and addressing its negative aspects. Finally, *ad hoc* gene correction protocols developed to correct mutations associated with specific genetic diseases are presented in the fourth section (Part IV).

I am passionate about gene correction because it gives us the tools for both repairing and mutating DNA, for discovering gene functions and for engineering new genetic variants. As Nobel laureate for gene targeting in mice, Mario Capecchi once said, “gene targeting gives us complete freedom in choosing which gene to alter and how to alter it.” The preparation of this book has been an exciting experience. I learned a lot from reading and reviewing the chapters. I think all the methods and protocols collected in this volume are a precious resource for the current and future gene “targeters”....there is still a long way to go!

The participating authors deserve great appreciation for the valuable contribution, effort, and patience they offered for the preparation of this volume. I am extremely thankful to all contributors. I would like to thank very much John Walker for his constant assistance and advice for this book. I am also grateful to all the staff at Springer and Humana Press for their work in assembling the chapters and producing this book.

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