
Preface

In vitro mutagenesis remains an essential tool for molecular biologists, biochemists, and metabolic engineers in performing both basic and applied research into gene and protein function. It is a cross-disciplinary research and development approach that has broad applications in many important fields. With the advent of ever more sophisticated investigative and analytical methods such as next-generation sequencing, emerging, powerful gene-editing techniques, and comprehensive data analysis software programs to process large data sets, the molecular biology field is witnessing a magnitude change in the understanding of gene and protein structural and functional relationships at the organismal level. With these powerful tools at researchers' disposal, it is now conceivable to design an in vitro mutagenesis strategy in a genetically manipulable organism, carry out the mutagenesis experiments in an efficient manner, generate, retrieve, and analyze the sequence data and impacted proteins and phenotypes in weeks or months instead of years, all with far greater insight into the understanding of the cellular and systems biology of the respective organism(s) under study. The molecular biologist's "toolkit" is in a sense now complete for designing, performing, and analyzing all manner of in vitro mutagenesis experiments with broad applications in basic research and for any conceivable commercial purpose—be it drug design, microbial cell factories development, gene therapy, or generation of model organisms, among many other applications.

In this volume on *In Vitro Mutagenesis: Methods and Protocols*, an important aim was to provide the beginner practitioner in the field and the more experienced molecular biologists, biochemists, and metabolic engineers alike a wide variety of updated, novel approaches to many powerful classical methods of performing in vitro mutagenesis such as transposon (Tn) mutagenesis, site-directed and random mutagenesis. Additionally, an important emphasis was placed on emerging, yet powerful, gene- and genome-editing and bioinformatics methods now being developed and implemented into experimental reality.

The volume is divided into seven sections: The first two sections describe detailed, novel methods for gene and genome editing of a broad cross section of the living world (e.g., mammalian, plant, viral, bacterial, and protistan systems using CRISPR/Cas9, TALEN, and Group II intron technologies). These editing technologies are enabling the facile genetic manipulation of a wide range of cellular systems in the study of fundamental metabolic processes. Importantly, they are rigorously being employed for the prospect and promise of attaining major scientific breakthroughs in human physiology and medicine. The next two sections describe (1) a variety of practical bioinformatics approaches for identifying mutagenesis targets in silico for the rational design of mutagenesis experiments and (2) a set of diverse, detailed in vitro Tn mutagenesis protocols for model microorganisms as well as for use in alternative, previously recalcitrant organisms (e.g., archaea) including genomic sequencing methods to rapidly and completely identify all the Tn insertion sites in a mutant library. The last three sections cover a wide variety of novel site-directed and random mutagenesis approaches aimed at gaining a better understanding of protein-protein and protein-cofactor interactions at the structural and functional levels along with their concomitant effects on an organism's cellular metabolism. Provided in these sections are specialized mutagenic PCR methods (e.g., is-epPCR, epPCR using heavy water, and single

primer PCR, et al.) and mutagenesis and cloning methods (e.g., MUPAC, SliP-SliCE, et al.). Several of these chapters also describe state-of-the-art bioanalytical techniques and methods used by experts in the field to allow a more thorough understanding of how the specific mutation impacted the experimental outcome. The rationale being that the beginner practitioner would be able to view the experimental process from conception and design to completion through follow-up data analysis.

In keeping with the theme of the Methods in Molecular Biology series, each chapter contains an extensive Notes section which provides and elaborates on specific experimental details, tips, and tricks and thus it is hoped will allow a more rapid, successful implementation of the method by practitioners in the field whatever their experience level.

I would like to thank the authors, who are all well-known experts in their respective fields, for their contributions and for allowing me to put together what I believe is a timely, practical, and comprehensive manual on in vitro mutagenesis methods and protocols that will be embraced by a broad range of practitioners in the fields of molecular biology, biochemistry, biochemical and metabolic engineering, biophysics, among other disciplines. Moreover, this volume on in vitro mutagenesis was specifically compiled with an emphasis on providing a highly accessible manual for current and future researchers—from the beginner practitioner to the advanced investigator—who routinely perform in vitro mutagenesis experiments on all types of cells at research institutes, academia, and industrial and government laboratories.

Lastly, I would like to thank all my past and current colleagues who have inspired me to keep learning and to pursue knowledge through hard work, persistence, and investigative scientific endeavors.

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In Vitro Mutagenesis

Methods and Protocols

Reeves, A. (Ed.)

2017, XV, 511 p. 96 illus., 52 illus. in color., Hardcover

ISBN: 978-1-4939-6470-3

A product of Humana Press