

METHODS IN MOLECULAR BIOLOGY

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Mycobacteria Protocols

Third Edition

Edited by

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Preface

The mycobacteria continue to pose a threat to global health, as well as being important biological systems to understand. In the years since the Second Edition of this book, research has continued into understanding these organisms and a number of new techniques have been introduced into our technical repertoire. The aim of this book is to expand upon previous edition, providing a selection of the newest methods, as well as some of the basic methods required for a mycobacterial research laboratory. There is an increased focus on translational methods, in particular those that can be applied to drug discovery, since there is a much larger, concerted effort in that direction.

The first six chapters deal with fractionation and analysis of macromolecules, from nucleic acids to proteins, complex lipids, and metabolites. Recent advances in sequencing technologies as well as the development of metabolomics approaches provide several new methods over previous editions. Chapters regarding whole-genome sequencing, transcriptomics analysis of gene expression, and transcript mapping are all beneficiaries of the new, lower cost, higher throughput sequencing techniques. Detailed and comprehensive protocols are provided for protein and lipid/glycolipid analysis using well-established methods; these are now complemented by a metabolomics chapter in which the complement of metabolites can be profiled.

Genetic manipulation underpins our ability to investigate the biology of the mycobacteria, and chapters cover the basics of electroporation, through to advanced genetic engineering to construct sophisticated recombinant strains (gene knockouts, knockdowns, and mutations) using either homologous recombination or recombineering.

Later chapters deal with model culture systems that can be widely applied to mycobacterial species and mutant strains. These include two models of hypoxia survival and newer approaches to analyze biofilm formation. In addition, methods to look at efflux and permeability as well as the powerful approach of studying single cells are described.

Several chapters deal with methods for characterizing novel antimycobacterial agents. Since bacteria can show tolerance or resistance to the same antibiotics depending on their physiological state, several alternative methods are presented to cover a variety of conditions. Chapters covering both growth inhibition of replicating organisms, killing of replicating and nonreplicating organisms and intracellular organisms are included.

Of all the mycobacteria, *Mycobacterium tuberculosis* has become the most studied, due to the large burden of global disease it causes. Since one of its characteristics is the ability to survive and replicate in immune cells, chapters cover a range of methods, from the “simple” macrophage infection model, and the neutrophil model, analysis of bead-phagosomes, through to infection of the model organism *Dictyostelium*. Finally, a sophisticated model to look at host-pathogen interactions using high content screening is presented.

We hope that this book will be a resource both to those working in the field and to newcomers. We thank all of our authors for their generous contributions, in the knowledge that sharing their expertise and wealth of experience will enable more rapid advances in the field.

Seattle, WA, USA

Tanya Parish
David M. Roberts

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