

Preface

The mycobacteria include a number of important human and animal pathogens and pose major problems worldwide in terms of global health and economies. Tuberculosis poses a significant threat to global health by infecting and killing millions annually. Leprosy has not yet been eradicated, and other infections, such as Buruli ulcer and opportunistic infections associated with immunodeficiency, are on the rise. For these reasons, the need for methods to study the biology of the mycobacteria and to improve diagnostic, therapeutic, and preventative reagents is still a priority.

It has been nearly 10 years since the first edition of *Mycobacteria Protocols*. The response to the first edition was both surprising and pleasing. Many readers commented on how the book had helped them with a tricky problem or allowed them to branch into new areas, and newcomers to the field were able to avoid many common pitfalls and progress quickly. Mycobacteria are difficult organisms to work with, but the availability of the tips and tricks developed by a multitude of scientists over many years has been received very positively. Within my own laboratory, this book has proved invaluable for neophytes. During the time since it was published, research into the mycobacteria has continued to expand, and the number of scientists studying these problematic bacteria has increased. New methods have been developed, and older methods have been refined, making a second edition timely.

In this second edition, we have tried to include a range of methods, from the basics of subcellular fractionation, strain typing, and determining minimum inhibitory concentrations (MICs) to more advanced methods using specialized growth conditions and whole genome, transcriptome, and proteome analyses. Although we cannot include all methods used in the modern research laboratory, we hope that this edition will provide a useful primer for new mycobacterial researchers and stimulate new avenues for established scientists.

In developing this edition, we have revised and updated some of the most used methods from the original version and incorporated a number of wholly new methods, some of which make use of our expanded horizons in the post-genomic age. Several methods are described for *Mycobacterium tuberculosis*, as this is now the most widely studied species, but can be easily adapted for other members of the genus.

Updated and revised methods include techniques for subcellular fractionation, notably the isolation of genomic DNA and RNA—techniques that are now more useful than ever given the availability of whole genome microarrays. Methods for identifying deletions in genomic DNA and for assaying genome-wide gene expression patterns are presented to make use of these fractions. Analysis of the proteome by two-dimensional gel electrophoresis is also included. These methods will allow the exploitation of the information afforded by the availability of the whole genome sequence for several mycobacterial species. Such a concerted sequencing effort has provided a plethora of information, although this can sometimes be overwhelming. *MycoDB* is an online resource designed to facilitate genomic analyses of *Mycobacterium* spp. and related genera, and a chapter explaining this powerful engine is included.

Mycobacteria have a characteristic lipid-rich cell wall. This outer layer is responsible for a number of their features, including the acid-fast property used to identify the genus. Cell wall composition has implications in many other areas, from immune recognition to insensitivity to antibiotics. A number of techniques are presented to allow the reader to analyze the composition of the cell wall including the complex lipoglycans, which include the immunomodulatory molecule lipoarabinomannan. Lipids plays an important role in mycobacteria, and methods for analyzing lipid biosynthesis and location as well as metabolism are detailed. The cell wall forms a strong barrier to many antimicrobials, and the use of transport assays to measure permeability is discussed.

Genetic techniques for manipulating the genome of mycobacteria have greatly improved since the past edition. Introducing recombinant DNA into mycobacteria is not as straightforward as for other bacterial species, so an updated version of the electroporation chapter is included. Much has happened in the plasmid field, and an updated review of the most commonly used vectors is given together with methods for using the only available temperature-sensitive plasmid, and two chapters deal with expression of genes in mycobacteria using inducible plasmid systems. Homologous recombination has been used to construct mutants for many years, and two methods are described here. Other methods for creating mutants have also been developed, and phage transposon mutagenesis is described. The identification of mycobacterial promoters and analysis of gene expression is a growing area of research, and a chapter that describes the use of two reporter systems, powerful tools for investigating transcriptional regulation in response to environmental signals, is included. A great deal of interest in identifying essential genes has arisen, and two techniques are provided for checking the essentiality of a given gene. The first method, CESTET, can rapidly identify essential genes. The second method, gene switching, can also be used to study the role of essential genes further.

The mycobacteria include several pathogens, so there is a need for strain identification in molecular epidemiology and genome variation studies. Methods for typing strains using insertion sequence, IS6110, and the newer

PCR-based method of analyzing repeat units (MIRU/VNTR) are given. The determination of MICs can be technically difficult, so protocols that can be used with clinical isolates and an Alamar blue-based method that could be applied to the development of novel inhibitors are included. A method for looking at molecular drug resistance using a scanning-frame oligonucleotide microarray is also included.

We have included a chapter describing the use of a chemostat to grow mycobacterial cultures under highly defined conditions, which is useful for studying environmental responses. One particular environment of interest to mycobacteriologists is the intracellular environment, as several mycobacterial species can survive and multiply inside eukaryotic cells. A model for this is provided in a chapter dealing with survival and gene expression in amoebae.

We hope that this book helps to promote and stimulate further research into these intriguing, important, and most fractious of bacteria. We would like to thank all the authors who have made this second edition possible and hope that these updated and new protocols will continue to serve the mycobacterial research community as a useful resource.

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