Preface

The aim of this book is to provide detailed protocols for studying the molecular biology of the pathogen *Mycobacterium tuberculosis*, and its interactions with host cells. As established mycobacterial laboratories move towards exploiting the genome, and laboratories with expertise in other fields apply them to mycobacteria, both traditional and novel methodologies need to be reviewed. Thus the chapters in *Mycobacterium tuberculosis Protocols* range from perspectives on storage of strains and safety issues to the application of the latest functional genomics technologies.

The last few years have been remarkable ones for research into *M. tuberculosis*. The most important landmark by far has been the completion of the genome sequence of the widely studied H37Rv strain (1). We can now predict every protein and RNA molecule made by the pathogen. This information is or will soon be enriched by the addition of genome sequences of other strains from the *M. tuberculosis* complex: a second strain of *M. tuberculosis*, *Mycobacterium bovis*, and the vaccine strain, *M. bovis* BCG. Valuable comparative data will also be provided by the genome sequences of *Mycobacterium leprae*, *Mycobacterium avium*, and *Streptomyces coelicolor*. Another recent milestone for *M. tuberculosis* has been the development of efficient mutagenesis methodologies, the lack of which has been a major handicap in functional studies. The new challenges to researchers are first to use this information and these techniques in combination with the battery of methodologies being developed around the world to exploit all genome data, so-called functional genomics research, which includes transcriptomics and proteomics. The second challenge is to integrate them with other disciplines of active research such as immunology, cell biology, and biochemistry. *Mycobacterium tuberculosis Protocols* incorporates both of these aspects in the methods described.

This book is aimed at people who are actively working on *M. tuberculosis.* However, there is much that will be relevant to work on other mycobacteria and on such phylogenetically related organisms as corynebacteria and streptomycetes. It is intended both for people with experience in handling *M. tuberculosis* and those who are new to the field.

The topics covered by the 24 chapters included are quite diverse. A major focus is the production of mutants, which plays a central role in functional studies. The recent successes in mutagenesis are reflected in the inclusion of four chapters (4–7) describing different strategies for both transposon mutagenesis and targeted allelic replacement.

Two of the most exciting recent technological developments make use of the genome sequence to allow us to look at the RNA and protein complements of the cell at a global level. Proteome analysis is described in Chapter 21, and the production and analysis of whole genome microarrays is described in Chapter 22. RNA is discussed in more detail elsewhere, with chapters on purification (Chap. 3), transcriptional start site analysis (Chap. 8), and quantitation using real-time PCR (Chap. 19).

Fractionation of the bacteria to look at protein carbohydrate and lipid components is described. The contributions focus on analysis of culture filtrates (Chap. 13), the capsule (Chap. 14), and lipids (Chap. 15). Cytological analysis of the bacteria allows the analysis of cellular properties in individual bacteria, and many of the major technological advances that have helped eukaryotic cell biology are beginning to be applied to bacteria (Chap. 9).

There is understandably enormous interest in how *M. tuberculosis* interacts with the host, and chapters discuss infection of macrophages both as a virulence assay (Chap. 17), and in order to understand the cell biology (Chap. 18). In addition, perhaps the most difficult topic to study, the persistence of bacteria, is addressed in Chap. 16.

The excitement of the post-genome era must not distract us from the fact that tuberculosis is a dreadful disease of which millions die each year, and control still suffers from difficulties in diagnosis. A relatively new method for detecting bacteria and identifying drug resistance using a cheap and sensitive phage-based method is described (Chap. 10). This method is phenotypic, in that the basis of the drug resistance need not be known. Genotypic methods are described that identify specific mutations by dot-blotting (Chap. 11) or real-time PCR (Chap. 19). Our understanding of the spread of bacteria has been revolutionized by DNA typing techniques, and the most up-to-date methodology for carrying out RFLP typing is in Chap. 12. DNA preparation from bacteria cultures and clinical isolates is discussed in Chap. 2.

Though most of *Mycobacterium tuberculosis Protocols* concentrates on laboratory methods, the genome sequence is a central resource for laboratory and bioinformatics research. A description of the main *M. tuberculosis* genome resources on the internet is therefore provided in Chap. 20.

Finally there are chapters providing basic but essential methods for work with *M. tuberculosis.* These are an up-to-date account of available cloning vectors (Chap. 1), how to store strains (Chap. 23). and last but by no means least, a discussion of some of the safety issues (Chap. 24).

This book aims to complement and update the earlier volume in this series, *Mycobacteria Protocols* (2). Some methods are deliberately complementary—for example, the computer analysis of IS*6110* fingerprints was described in the earlier book, whereas the production of the fingerprints is described here. Several diagnostic methods included in the earlier volume complement those presented here. Other topics in the earlier book that are relevant to tuberculosis research are those on pulsed field gel electrophoresis, preparation of cell-free extracts and cell wall fractions, the use of mycobacteriophages, and the analysis of gene expression using reporter genes and RT- and RAP-PCR.

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