Preface

Over 70 years ago, microbial genetics led to the molecular revolution in biology, providing powerful new methods for exploring the fundamental mechanisms of life. Beginning in the mid-1970s, the development of molecular cloning coupled with the characterization of transposons led to a second revolution in molecular genetics, providing a set of indispensable tools that facilitated the expansion of basic genetic selections and screens from the *E. coli*, *Bacillus*, and *Salmonella* paradigms throughout the microbial world. Recently, another revolution in molecular genetics has been stimulated by the sequence determination of hundreds of complete genomes in combination with methods to determine RNA and protein expression levels as well as a complete library of sets of interacting proteins and genome-scale three-dimensional protein structure determinations. These new approaches provide insights into how each of the multiple different cell components changes in response to a particular stimulus—a Gestalt perspective called systems biology. However, confirming the predictions from whole genome approaches requires genetic analysis to verify the roles of particular gene products. Combining systems biology and genetic approaches will provide novel insights into basic biological processes and into the role of microbes in the environment, health, and disease.

Transposons and bacteriophage continue to play a key role in the dissection of fundamental processes, whether at the single pathway or whole genome scale. This volume describes some of the advances in bacterial genetics that have expanded the uses of transposons and bacteriophage in genomic engineering. The basics of genetic analysis using transposons are covered in Chapters 1, 2, 3, and 5. Transposons provide efficient methods of constructing mutations and selectable linked genetic markers, moving mutations into new strains, facilitating mutagenesis of defined regions of the genome (Chapter 6), and constructing fusions (Chapter 13). In addition, because multiple copies of a transposon in a cell provide regions of genetic homology that can be placed at any desired position, transposons can be used to select for recombination events that yield defined deletions and duplications (Chapter 7). The ability of transposons to generate large chromosomal duplications allows for the identification of all essential genes in an organism and the isolation of reporter fusions to characterize the expression of these genes (Chapter 4). In addition, transposons have been modified for the construction of specific reporters or the introduction of small peptides for the probing of three-dimensional structure and protein location or topology in the cell (Chapters 9 and 12). Uses of

transposons to tag specific proteins with protease sites or with antigen sites are useful for controlling and measuring levels of given proteins under any condition (Chapters 8, 9, and 12).

With the acquisition of whole genome sequences, it is now possible to characterize transposon mutant libraries on a genomic scale. Methods are described for creating and maintaining such libraries as well as combining genomic microarray analysis to study the impact of the transposon insertions on whole genome gene expression under any set of conditions desired (Chapters 10, 11, and 12). Transposons can also be utilized in combination with inducible promoters to probe whole genomes for genes that, when induced, have positive or negative regulatory effects on the system under investigation (Chapter 14).

New advances in bacteriophage technologies that have virtually eliminated the artifacts of working with multicopy plasmid vectors are described. Phage recombination systems have been harnessed to allow the use of synthetic oligonucleotides for engineering mutations or fusions for the study of gene regulation, protein topology, or cell biology (Chapters 15 and 16). Phage systems have been modified that allow the characterization of chromosome structure (Chapter 17), for studying protein–DNA, protein–protein, and protein–RNA interactions (Chapter 18), and for the manipulation of specific segments of the bacterial chromosome (Chapter 19). Advances in phage metagenomics (Chapter 20) allow the identification of millions of novel proteins from diverse environments around the globe, whether or not the phage or host can be grown in the lab (Chapter 20).

In short, microbial genetics remains a vibrant and important field that has both been enriched by new methods of systems biology and provides useful tools for systems biology. Transposons and phage continue to provide invaluable new tools for dissecting the structure, function, regulation, and physiology of microbes.

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