

be so all-inclusive perhaps leads to a watering down of focus. For example, within the subsets, there is a good coverage of the Gram-negative bacteria, although it is surprising that *E. coli* is only represented as part of the first chapter. There is less broad coverage of Gram-positive organisms. Indeed, it is quite a surprise that no chapter exists on *Bacillus subtilis*, perhaps the best-explored of the bacterial organisms in the context of molecular stress response research (particularly if one considers starvation and sporulation). It is acknowledged that the focus is to be on pathogenic organisms, and thus *Bacillus cereus* is substituted instead. A major omission is that of *Staphylococcus aureus*, one of the most prominent pathogenic bacteria anywhere in the world, and one with a strong research base with regards to stress behaviors and response.

In terms of topics, one might be surprised to find that no chapter focuses on the response of microorganisms to challenge by antimicrobial agents. Given the extensive material available on this topic, and the undeniable importance to human health, such an inclusion would have made a nice complement to the pathogenic and environmental content. Indeed this could have incorporated the evolutionary origins of antibiotics and bacterial response, for example, the soil, thus pulling in *Bacillus subtilis* and the wealth of knowledge for this bacterium.

In terms of the individual chapters, there is some variability with regards to how comprehensive they are. Whilst all chapters are strong, some are a little thin for their respective topics, with others containing no tables or figures, which are always helpful in review texts. Also, although this is a rare occurrence, some of the figures are of low quality and resolution, making them hard to see. Another consideration is that many of the chapter titles are generic, and ultimately not very informative, for example 10 of the 17 chapters are entitled "Stress response in ...". More thought could perhaps have been given to titling these so as to provide a diverse coverage of topics and an easier way for the reader to navigate the volume. Additionally, it would have been helpful to include a table of tables, and table of figures in

the contents section to help navigate this volume.

Ultimately, this is a very nice volume, with an ambitious and challenging subject matter. The diverse array of organisms and contributing authors make for a well-rounded and enjoyable reference tool. One would predict that this would be of significant interest to any student of microorganisms, and their interaction with, and response to, complex and stressful environmental conditions.

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Methods in Molecular Biology 772: Molecular Methods for Evolutionary Genetics

Edited by Virginie Orgogozo and
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Humana Press, Totowa 2011, XVI + 503 pp.,
hardcover, \$ 139.00.—ISBN 978-1-61779-227-4

Technical advances have traditionally been a key driver of scientific discovery. In the field of molecular evolution, the application in the mid-1960s of electrophoresis to questions about amino acid polymorphism in a single protein enabled the first molecular evidence of genetic variation. In the 1980s, the advent of DNA sequencing revealed that the amount of genetic variation at a single locus was far greater than expected. Since 2000, the expansion of DNA sequencing to encompass whole genomes has led to models of molecular evolutionary networks containing thousands of genes that are intended to more accurately reflect the action of natural selection on existing genetic variation in a population.

The current toolkit for genome-level studies capable of addressing both molecular biology and evolutionary genetics questions is described in a new volume from the well-respected *Methods in Molecular Biology* series—*Molecular Methods for Evolutionary Genetics*. This volume is edited by two newly independent researchers actively working in the field of

molecular evolution, Dr. Virginie Orgogozo a CNRS Junior Group Leader at the Institute Jacques Monod and Dr. Mathew V. Rockman, an Assistant Professor at New York University.

The book contains 28 chapters organized into six sections that focus on the different phases one would move through when conducting an evolutionary genetic analysis of a new genome. The first two sections "Characterizing the Genome" and "Targeting Regions of the Genome" contain chapters detailing molecular biology techniques that can be employed to characterize unexplored genomes, such as determining genome size and cytology, generating genomic libraries, normalizing mRNA pools, and sequencing the transcriptome. The next two sections "Measuring Genetic Diversity" and "Obtaining Candidate Gene Sequences", describe methods for assessing the diversity of an uncharted genome through microarray analysis, single-nucleotide polymorphism (SNP) discovery and genotyping by next-generation sequencing, and isolation of DNA sequence variants or novel sequences via PCR. The final two sections "Analyzing Candidate Gene Transcripts" and "Testing Candidate Genes and Candidate Mutations" outline methods for examining the biological and evolutionary implications of genetic variability detected in an unfamiliar genome. Included in these sections are chapters on quantifying transcript levels with RT-PCR and pyrosequencing, in situ hybridization to fixed tissue, evaluating gene regulation with reporter constructs, genomic transgenes and gene knockdown techniques.

All of the chapters are organized with the standard *Methods in Molecular Biology* format, which consists of an introduction, materials list, methods description and notes. The introduction explains the purpose of the technique and provides parameters for its use. Related techniques that might be better suited for a particular situation are also discussed. The methods section provides the rationale for each step. Of particular value is the technical trouble-shooting information found in the notes section.

We describe a few chapters from each section as examples. In the section on "Characterizing the Genome", Mike Quail

and colleagues report methods for creating and manipulating a fosmid genomic library. Chapter 3 provides a detailed protocol for generating the library and for isolating clones of interest from the library. Not only is the procedure rigorously spelled out, but the important steps necessary to assess the quality of the library in real time as well as its proper storage once completed are included. In Chapter 4, the authors provide equivalent detail in their recitation of the steps needed for subcloning, sequencing and then assembling large-insert genomic clones.

Within the section on "Obtaining Candidate Gene Sequences", Michael Lang and Virginie Orgogozo discuss (Chapter 14) how to isolate homologous sequences with degenerate primers and amplify them by PCR. As one would guess, the most important steps in this process are choosing the template region and designing the primers. In the methods section, these steps are discussed in detail. Having done a fair amount of PCR with

degenerate primers ourselves, we recognize in this chapter many of the parameters we employed intuitively. By codifying the rules for primer design, the authors provide a useful guide for troubleshooting a failed experiment. In addition, the authors include a nice explanation of PCR chemistry that should be valuable for training young researchers.

Chapters in the last two sections cover a broad range of techniques and address questions that were difficult to answer previously. A nice example of this is Chapter 18 in which Patricia Wittkopp describes a method for measuring *cis*- and *trans*-regulatory differences between genotypes through pyrosequence-derived measurements of allele-specific mRNA abundance. The chapter opens with a well-written introduction to pyrosequencing, and the instructions for experimental design and creating appropriate primers are technically valuable and provide the reader with a theoretical foundation for interpreting the results.

Notwithstanding the breadth of techniques described in this volume, the field of genomic analysis has exploded to such an extent that no reasonably priced book can be exhaustive. From our perspective, a chapter on phylogenetics as a tool for assessing homology in multigene families and another describing technologies for analyzing epigenetic features of genomes would have been welcome. This minor shortcoming does not deter us from our recommendation that this book provides a useful compendium that students, postdocs and new investigators would be well advised to keep by their lab bench, and that experienced researchers moving into the field of genomic analysis should have on their bookshelf.

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DOI: 10.1002/cbic.201200656