Preface

We are excited to present this collection of protocols for genetically analyzing bacteria and their viral predators, phages. Bacteria and phages are ubiquitous on our planet and can be found in the air we breathe, on our skin, and in our intestines; in water, soil, and the roots of plants; and deep down into the Earth's crust. Bacteria are both friends and foes, and they warrant continued study to further our understanding of biological processes, to develop biologics, and for many other medical, biotechnological, and industrial applications.

Genetic analysis of phages and bacteria exploded in the twentieth century and was instrumental in building the foundations of molecular biology and molecular genetics. The pace of discovery has not slowed since and has even been hastened by whole-genome sequencing and computational tools. Underpinning these breakthroughs are carefully developed methods and protocols, which have been improved and broadened over the years by talented scientists in many laboratories, allowing hypotheses to be tested and discoveries to be made. This growth has been made possible by those skilled in these experimental arts passing these tools on to bright young minds, so that new generations of researchers can continue and advance research in the field.

Here, we provide a collection of some of the methods and protocols that have served as the foundation for great advances in the field of bacterial genetics. Although this protocol collection is designed primarily for scientists with access to modern facilities, equipment, and a reasonable supply budget, several of the protocols require very little by way of sophisticated equipment and can be carried out in even the most basic laboratory settings. This collection of protocols, also available online at https://cshprotocols.cshlp.org, starts with fundamental procedures in molecular genetic research. It then covers isolation and genome sequencing of novel species of bacteria. Next, it delves into selection of phenotypic mutants and identification of the underlying mutations using genome resequencing. Protocols for high-throughput transposon mutagenesis as well as for using transposons to generate gene fusions are described, along with procedures for making gene deletions, defined mutations, or gene fusions by plasmid recombination or via the λ Red "recombineering" technique. Finally, genome engineering of bacteria and phages using two different CRISPR technologies is described. The protocols herein presented are described for various model organisms, including *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Vibrio cholerae*, and *Staphylococcus aureus*, along with some of their phages. However, with some tweaking, many of the protocols can also be applied to other bacteria and their phages. Protocols for whole-genome sequencing and annotation can be applied to any culturable bacteria or their phages. These protocols, however, cannot be applied to nonculturable bacteria nor are they easily applied to culturable bacteria for which DNA transformation procedures have yet to be developed, although the conjugation method described in some of the included protocols has the potential for DNA transfer to such organisms.

We thank all the scientists who contributed to this protocol collection, including Cecilia Silva-Valenzuela, Miriam Ramliden, Lauren Shull, and the co-authors listed on the protocols. We are grateful for their excitement, careful work, and thoughtful contributions to the design and implementation of the different protocols used in the various editions of the Advanced Bacterial Genetics course held at the Cold Spring Harbor Laboratory, which served as the basis for this collection. We thank David Stewart, Barbara Zane, Rachel Lopez, and many others at Cold Spring Harbor Laboratory for making the course and our development of these protocols a success. We thank the students of the course, who through their trial and error, helped us fine-tune these protocols. We thank student Landon Getz, for the beautiful picture of bacterial colonies that adorns this book cover. We would also thank Maria Smit, Inez Sialiano, Shannon Coleman, David Hatton, Christin Munkittrick, Kathleen Bubbeo, Alejandro Montenegro-Montero, Denise Weiss, and Richard Sever at

Cold Spring Harbor Laboratory Press for their help and enthusiasm for this book. Last but not least, we would like to thank all the teams of Instructors who taught the Advanced Bacterial Genetics course before us, for setting a standard that inspired and motivated us all along this endeavor.

We hope that this protocol collection will be useful for a wide range of researchers who study bacteria and phages, and trust that the community will continue to enhance and broaden these methods and make them public knowledge.

Andrew Camilli
Angelika Gründling
Lionello Bossi

GENERAL SAFETY AND HAZARDOUS MATERIAL INFORMATION

Cold Spring Harbor Laboratory Manuals should be used by laboratory personnel with experience in laboratory and chemical safety or students under the supervision of such trained personnel. The procedures, chemicals, and equipment referenced in these manuals are hazardous and can cause serious injury unless performed, handled, and used with care and in a manner consistent with safe laboratory practices. Students and researchers using the procedures in this manual do so at their own risk. It is essential for your safety that you consult the appropriate Material Safety Data Sheets, the manufacturers' manuals provided with the relevant equipment, and your institution's Environmental Health and Safety Office (hereafter referred to as safety office), as well as the General Safety and Disposal Cautions in the Appendix for proper handling of hazardous materials. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in its manuals and has no liability in connection with the use of these materials.

All registered trademarks, trade names, and brand names mentioned in this book are the property of the respective owners. Readers should please consult individual manufacturers and other resources for current and specific product information.

Appropriate sources for obtaining safety information and general guidelines for laboratory safety are provided in the General Safety and Hazardous Material Information Appendix.