Preface

Why do we still study *E. coli*? One attraction of working with such cells is that they represent a simplified system. The possibility of harvesting a large number of cells in a short time, and the advantage offered by a haploid organism containing only one chromosome and which can double every 20 minutes, have prompted many investigators to use *E. coli* for genetics research. Their work has considerably increased our knowledge of this organism and has resulted in the development of numerous specialized techniques, many of which we use in this manual.

Most importantly, there remain a vast number of basic problems in the field of cell biology which are as yet poorly understood in even a relatively simple system like *E. coli*. DNA replication, recombination, and repair are examples. Much about the mechanism of transcriptional (mRNA) control is unclear, for instance, positive control. The question of how the synthesis of ribosomal and transfer RNA is controlled is still largely unanswered. Many details of protein synthesis have not yet been elucidated, and the DNA and RNA sequences coding for the initiation and termination of transcription and translation are just now being deduced. The problem of controlled degradation of mRNA and of proteins still is mostly unsolved, and the study of membranes and transport may also be dependent on systems such as *E. coli*. Finally, there remain many unresolved aspects of intermediary metabolism.

How can bacterial genetics help solve these problems? The answer to this question is the subject of this manual. The basic approach that this field offers is the isolation of mutants. The discovery of new control systems, the tailoring of enzymes by genetic manipulation, and the definition of genes involved in biochemical pathways and processes are all direct results of this approach. Therefore, much of the text describes methods for the induction, isolation, characterization, and mapping of different types of mutations.

To facilitate the teaching of experimental molecular biology, we have compiled a series of experiments which can be done on a class basis and which cover many of the areas of modern bacterial genetics. Many of these experiments have been performed by student groups, such as the summer Bacterial Genetics Courses at Cold Spring Harbor. We use the *lac* operon for illustration often, and a review text, *The Lactose Operon* (Cold Spring Harbor Laboratory, 1970) has recently been published which provides a valuable summary of work on this basic system. We were fortunate to able to draw on the excellent experimental manual by Clowes and Hayes (John Wiley and Sons, Inc., 1968) which served as a model for much of this book.

We have also tried to bring together into one volume as many recipes and methods as possible to enable investigators to use this text as a research handbook. Although the *lac* system is used for demonstrative purposes throughout part of the manual, almost all of the methods and techniques described are general. Thus, the description of indicator plates, mutagenesis, Hfr crosses, strain construction, hybridization, and enzyme assays are applicable to a wide variety of systems. Also, we hope that compiling these techniques will enable investigators not thoroughly acquainted with genetic manipulations in *E. coli* to form strategies for building strains and isolating mutants. One of the recent advances in bacterial genetics has been the development of techniques for incorporating bacterial genes into the DNA of certain phages. These specialized transducing phage are then used to provide DNA greatly enriched for the specific gene of interest. Experiments utilizing current methods for isolating these phage are presented in detail in this manual.

We have attempted to arrange these experiments in order of increasing difficulty and have tried to introduce the concepts of some of the later experiments in earlier sections. For instance, Experiment 42 (The Isolation of *trp-lac* Fusion Strains) is a series of steps, each of which has been covered previously. The object of the experiment is to isolate strains in which the *lac* genes are under the control of the *trp* operon. First, phage-resistant mutants are isolated and examined on lactose indicator plates. Recombination and complementation tests are then used to determine the end points of the deletions. Finally, Hfr crosses and F′ transfers are employed to prepare and test *trpR* derivatives of the fusion strains. (This experiment was successfully performed by different groups of 20 students at Cold Spring Harbor in 1969 and 1970.) We would like to emphasize, however, that there is no mandatory order to these experiments. We have included many more experiments in this manual than could possibly be accomplished in a one-semester course. This gives the students and instructors a large freedom of choice in selecting experiments and planning courses. For instance, Unit VI is certainly optional since this requires special equipment which may be unavailable in some laboratories.

In order to facilitate the use of this manual, we have made available strain kits containing the 79 strains described here and 5 lysates in small, 1-dram, agar-filled stab bottles. We also include in each kit a precision-made device, described in the Appendix, which is used for interrupted matings. Kits can be obtained from Cold Spring Harbor Laboratory for $100 to cover costs of handling, packaging, and mailing.