Preface

In the past decade, we have witnessed a revolution in the fields of molecular genetics and biochemistry. This revolution has not been the result of a single new development in instrumentation or a theoretical breakthrough, but rather due to the application of a variety of techniques collectively referred to as "Recombinant DNA Technology." This technology involves the *in vitro* modification and recombination of genetic material from different organisms to create new gene combinations.

The purpose of recombinant DNA research is to study gene structure and function, and, in many instances, to produce gene products that are otherwise difficult to obtain in pure or large amounts. Due to the broad spectrum of biological problems amenable to the recombinant DNA approach, the scientific literature is filled with exciting and significant reports describing new aspects of gene regulation, the organization of the eukaryotic genome, and the synthesis of mammalian proteins in bacteria. As a consequence, there has been a growing demand, among scientists and students alike, for the knowledge and training needed to apply this experimental approach to their own research projects.

In the fall of 1979, my colleagues and I at the University of California, Davis, offered a ten-week course entitled "Advanced Molecular Genetics Laboratory." The participants in this course included both graduate students and undergraduates with diverse backgrounds in the biological sciences. The purpose of this course was to acquaint these students with the concepts and techniques involved in recombinant DNA research, and their application to the genetic analysis of the arabinose and histidine operons of *E. coli*. These particular operons were chosen because of the wealth of information already available on their regulation and genetic organization. Furthermore, they represent interesting examples of positive and negative mechanisms for regulating gene expression. In response to the heavy demand for copies of laboratory handouts and protocols, we decided to make this material available in published form.

This manual is not intended to be a "cookbook" describing all recombinant DNA techniques currently available, but rather to provide the basic laboratory experience to allow the student to progress to more

advanced experiments. The laboratory exercises have been arranged so as to build on information covered in the preceding exercise. However, each exercise is presented in the context of a chapter, which discusses the theoretical and practical aspects of the experiment. Therefore, each chapter represents a module that can be used to perform the exercises, independently from the other chapters. The exercises are designed to instruct the students in the techniques of transformation, ligation, use of restriction enzymes, and the purification and analysis of DNA. Since our course has attracted students with varied backgrounds and a wide range of interests, the introductory sections to each chapter have been written in such a way as to be intelligible to those with a general knowledge of either microbiology, genetics, or biochemistry. The manual includes extensive appendices where recipes, reagents, buffers, and additional protocols can be found. In short, these features make the manual not only useful for laboratory instruction, but also as a reference book for individual research interests. Upon completion of these exercises, the student or researcher should have the necessary skills and understanding needed to proceed to more advanced and specialized methodologies.

All of the experimental procedures described in this manual have been performed by the authors and have been found to yield clear and reproducible results. Protocols have been carefully written so that the exercises can be performed in a minimum amount of time by the inexperienced experimentalist. Furthermore, the experiments utilize only a small number of commercial restriction enzymes and common laboratory equipment. Since these experiments involve the formation of recombinant DNA molecules from organisms that exchange genetic material naturally, they are exempt from the National Institutes of Health Guidelines on Recombinant DNA Research. However, in accordance with the Guidelines, these experiments should be performed in a P1 physical containment facility using the appropriate laboratory practices.

Due to the broad scope of the recombinant DNA field, it is impossible for us to cover all aspects of molecular cloning technology. For this reason we have focused our attention on plasmid vector systems and bacterial genes. We believe that these experiments are representative of many of the molecular cloning experiments currently found in scientific literature. We hope that this manual will fill the void that presently exists between the scientific literature and existing laboratory manuals.

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