Gene Cloning

*The Mechanics of
DNA Manipulation*

Outline Studies in Biology

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Gene Cloning

The Mechanics of DNA Manipulation

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Preface

This book was originally conceived in the form of a second edition of a volume published in 1980 in Chapman and Hall's 'Outline Studies in Biology' series and entitled *Genetic Engineering – Cloning DNA*. It very rapidly became apparent that with the impact of recombinant DNA techniques being felt in so many areas of biology, it was going to be difficult if not impossible to keep the book within the space confines of these little monographs. The stays were therefore loosened and the book expanded comfortably to its present size. I hope that this extra space has allowed me to clarify sections of the text that were 'heavy going' in the earlier version. The extra space has certainly allowed me to cover topics that were not mentioned at all in the earlier book. These are primarily to be found in Chapters 7 and 8, which cover the rapid advances that have been recently made in the use of plant and animal cells as hosts for recombinant DNA molecules. The development of other vectors has certainly not stood still over the past four years. This has necessitated a thorough revision of Chapters 3 and 4, which deal with bacteriophage and bacterial plasmid vectors. Numerous techniques for *in vitro* mutagenesis have now been tried and tested allowing me to give comprehensive coverage of this area in Chapter 2 along with the biochemical techniques used to construct recombinant DNA molecules. Readers with some background knowledge of the approaches to gene cloning will be able to go straight to a part of the book in which they are specifically interested. On the other hand, undergraduate readers with little background knowledge should benefit from studying the first chapter in which the basic principles of gene cloning are expounded.

The topics covered by the book are taught in a four week course, 'Genetic Engineering', given for final year undergraduates studying Molecular Biology at Imperial College. The book is intended for use in such advanced undergraduate study and also for graduate school coursework. I hope that it will be read as well by research workers wanting a general overview of the subject. When I first reviewed the subject of Gene Cloning in an article written in late 1975, I cited 38 papers which covered the whole field. Nowadays, it is difficult to find any paper dealing with the molecular biology of Nucleic Acids that does not use DNA cloning technology. Consequently it has been difficult to be selective in providing references. I hope that students will not feel overburdened by the references that I have given and that researchers will not feel neglected if their particular area has not been covered.

Preparation of the manuscript has taken considerable time and I am grateful to my research students and other colleagues for their tolerance of my involvement in the project. Finally I am grateful to Jean Beggs, Conrad Lichtenstein and Peter Rigby of Imperial College and Bill Brammar of Leicester University for their helpful comments on the manuscript at various stages of its evolution. Finally, I thank Martin Eilers, Manfred Frasch, Herbert Jäckle and Harry Saumweber of the Max Planck Institut für Entwicklungsbiologie in Tübingen for their help in reading the proofs.

Imperial College

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Cover picture

The photographs on the cover illustrate the procedure of colony hybridization explained in Section 1.2 (see also Fig. 1.1). The upper photograph shows a 'master' plate on which there are colonies of *Escherichia coli* containing recombinant plasmids spotted out to form a grid. The master plate has been used to 'print' out a number of replicas onto nitrocellulose filters. These have been incubated on nutrient agar allowing new colonies to grow in identical positions to those on the master plate. The nitrocellulose filters are later removed, and the DNA in the colonies denatured and immobilized on the filter using procedures described in Section 1.2. The filters are incubated with a radiolabeled nucleic acid probe which hybridizes to any complementary nucleic acid sequences in the colonies. The unhybridized radioactivity is washed away and the position of hybridization revealed by autoradiography. The lower photograph shows such an autoradiograph in which the probe has hybridized to DNA from two of the colonies. This particular experiment was carried out by Sarah Millar.