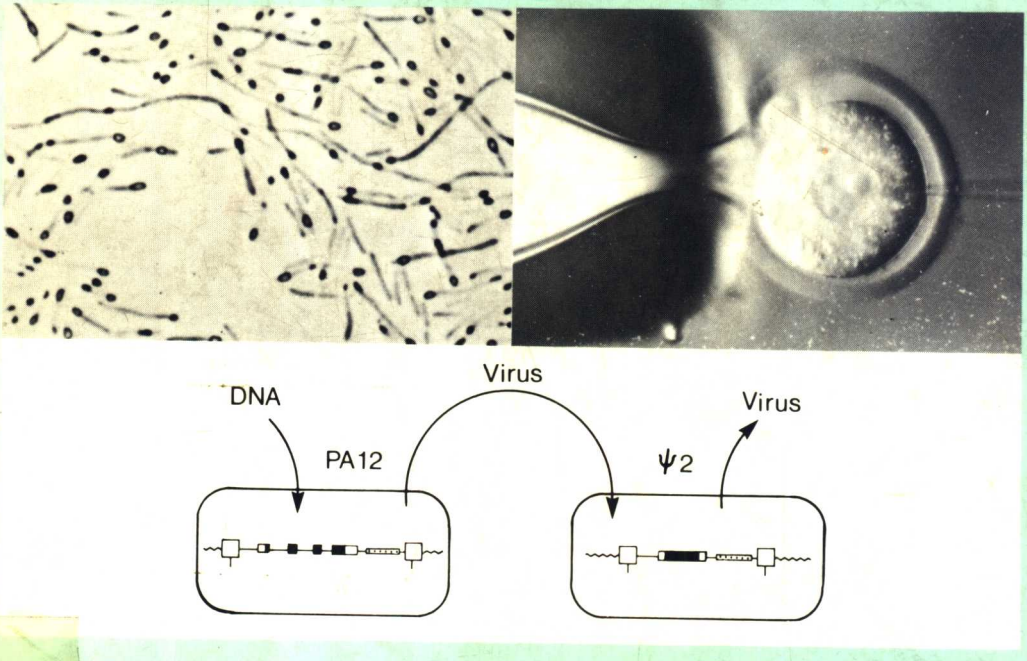
DNA cloning
Volume III

a practical approach

Edited by
D M Glover



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Cover illustration. The design for the cover was based on Figure 3 Chapter 4, showing a phase contrast micrograph of *Escherichia coli* HB101 cells producing prochymosin; Figure 10B Chapter 10, showing microinjection and consequent swelling of the male pronucleus; and Figure 5A, Chapter 9, showing a schematic representation for the generation of virus stocks.

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Preface

This is the third volume in this series describing DNA Cloning techniques, and as such is testimony to the pivotal position that these techniques now occupy in Molecular Biology. It was intended that the volumes complement and extend existing manuals describing the techniques of cloning DNA, especially the ubiquitous *Molecular Cloning* by Maniatis, Fritsch and Sambrook (Cold Spring Harbor Laboratory Press, New York, 1982). Their manual describes all basic cloning techniques and is referred to by most of the authors in this volume as it was in the first two volumes. The main theme of the first volume of *DNA Cloning* was the ongoing development of *Escherichia coli* as a host organism for a number of cloning systems. The second book looked at the diversity of other host/vector combinations that are used alongside *E. coli* to clone and express genes in prokaryotic and eukaryotic cells. The contents of this third volume are themselves diverse, and cover a variety of techniques for cloning and expressing DNA molecules. As with all laboratory oriented texts, some readers may well first require an introductory overview. As before, I recommend *Recombinant DNA: A Short Course* by Watson, Tooze and Kurtz (Scientific American Books, New York, 1983); *Principles of Gene Manipulation* by Old and Primrose (Blackwell, Oxford, 1985); and my book, *Gene Cloning: The Mechanics of DNA Manipulation* (Chapman and Hall, 1984).

The first chapter of the present volume covers the applications of plasmids containing promoters that are only recognized by RNA polymerases encoded by certain phages. These provide a means of synthesizing radiolabelled probes for several powerful types of analysis of nucleic acids. One set of cosmid vectors containing these promoters is examined in the second chapter. These vectors have been designed to facilitate walking along the chromosomes of higher eukaryotes. The phage promoters are positioned so that radiolabelled probes can be synthesized from the terminal regions of the inserted DNA, and subsequently be used to allow the isolation of overlapping cloned DNA segments. An alternative means of screening cosmid libraries appears in Chapter 3, which describes how cosmids can be selected genetically by homologous recombination with a probe plasmid *in vivo*. The latter half of the book focuses upon the expression of cloned genes. Many mammalian proteins have been expressed at high levels in *E. coli*, where they often form insoluble inclusion bodies, making the protein difficult to recover in a native form. Approaches to overcoming this and related problems are discussed in Chapter 4. The degradation of proteins of higher eukaryotes in *E. coli* can often be prevented by directing their synthesis as fusion proteins. These fusion proteins, in which the bacterial moiety is usually β-galactosidase, can be used as immunogens in order to raise antibodies against the eukaryotic segment. Two chapters describe how such fusion proteins can be used; one concentrating upon the production of antisera, and the other on monoclonal antibodies. Attention then turns to eukaryotic expression systems; first in a chapter devoted to the expression of foreign genes in yeast, and then in three chapters that examine mammalian cell systems. The first mammalian system that is described utilizes vectors which incorporate a gene that can be induced to amplify in order to overcome the toxic effects of a drug included in the culture medium. The cloned gene is also amplified and is consequently expressed at high levels. Retroviral vectors, described in the penultimate chapter, are finding

widespread applications. Rather than dwelling upon the more specialized applications of these vectors, this Chapter describes the experimental principles of handling the vectors and their use as general purpose expression vectors. The final chapter in the book describes the approach that has so far had the most success as a means of introducing genes into the whole mouse; microinjection of the fertilized egg. This route is only one of several possibilities as a means of achieving this end, and perhaps these other approaches will be covered in other books in this series.

It can be seen from these three volumes that DNA Cloning techniques have made their impact upon most areas of biological research. Whilst the books reflect the current state of the technology, it is impossible to give definitive accounts of many of the techniques which are continuing to evolve over the years. I hope, nevertheless, that the essential experimental principles can be gleaned from this volume. The success of the book will be judged by whether it finds its way, tattered and torn, onto laboratory benches. I hope that it will and that the methods described in it will be useful to the Molecular Biology community. Finally, and most importantly, I would like to thank all the authors for their hard work.

David M.Glover

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Abbreviations

ADA	adenosine deaminase
AFP	alpha fetoprotein
AIDS	acquired immune deficiency syndrome
bGH	bovine growth hormone
BPV	bovine papilloma virus
BSA	bovine serum albumin
CAT	chloramphenicol acetyltransferase
CEA	anti-carcinoembryonic antigen
c.f.u.	colony forming units
CIP	calf intestinal phosphatase
dCF	deoxycoformycin
DEPC	diethyl pyrocarbonate
DHFR	dihydrofolate reductase
DIC	differential interference contrast
DMs	double minutes
DOC	deoxycholic acid
DPD	dimethyl-pimelimidate dihydrochloride
DTT	dithiothreitol
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
ES	embryonic stem cells
EtBr	ethidium bromide
FSH	follicle stimulating hormone
GS	glutamine synthetase
hCG	human chorionic gonadotrophin
HSRs	homogeneously staining regions
IGF	insulin-like growth factor
IPTG	isopropyl β-D-thiogalactosidase
KSCN	potassium thiocyanate
LPS	lipopolysaccharide
LTRs	long terminal repeats
2-ME	2-mercaptoethanol
MLP	major late protomer
MLV	murine leukaemia virus
Mo-MLV	Moloney murine leukaemia virus
Mo-MSV	Moloney sarcoma virus
MSX	methionine sulphoximine
ORF	open reading frame
PBS	phosphate-buffered saline
p.c.	post coitum
PDGF	platelet-derived growth factor
PEG	polyethylene glycol
PMSF	phenylmethylsulphonyl fluoride
RNAsin	ribonuclease inhibitor
RIA	radioimmunoassay
RSV	Rous sarcoma virus
SDS	sodium dodecyl sulphate

SDS-PAGE	polyacrylamide gel electrophoresis in the presence of SDS
SOD	superoxide dismutase
TBS	Tris-HCl, NaCl and EDTA
TCA	trichloroacetic acid
t-PA	tissue-type plasminogen activator
VNC	vanadyl nucleotide complex
xyl A	9-D xyloguanosyl adenine
YIp	yeast integrating vectors

Contents

CHAPTER 1

Application of plasmids containing promoters specific for phage-encoded RNA polymerases

PETER F.R.LITTLE and IAN J.JACKSON

1. SCOPE OF THE CHAPTER

This chapter provides methods for synthesizing and using RNA made *in vitro* with SP6, T7 and T3 phage-encoded RNA polymerases. We have concentrated primarily upon the use of radiolabelled probes for the routine analysis of nucleic acids and have not attempted to cover, in anything other than broad outline, more specialist uses such as *in situ* hybridization, anti-sense mRNA and translation of products. We have attempted to provide the key references in these areas that will allow a reasonable appreciation of technical possibilities.

2. BACTERIOPHAGE ENCODED RNA POLYMERASES

2.1 **Background**

It has been known for many years that a variety of *E. coli* bacteriophages encode RNA polymerases that are capable only of transcribing particular promoters contained on the phage DNA. T7 and T3 phages were early examples (1) and more recently the phage SP6 of *Salmonella typhimurium* was shown (2) to have a polymerase with similar properties.

Phage-encoded RNA polymerases differ in many respects from their host polymerases. They are generally small (90 – 100 000 daltons), monomeric and have very limited but none the less highly specific promoter requirements. In contrast, *E. coli* RNA polymerase is large, heteromultimeric and capable of initiating RNA synthesis from a wide range of promoter and promoter-like sequences (3). The practical consequence of these differences is that it is possible to use phage encoded RNA polymerases to initiate RNA synthesis *in vitro* and generate specific single stranded RNA molecules. This cannot easily be achieved with the *E. coli* enzyme.

The first enzyme to be systematically used for the preparation of RNA probes was SP6 RNA polymerase (4). This was primarily because the enzyme was exceptionally stable and could be isolated in high yield by simple procedures, in contrast to the enzymes from T7 or T3. These latter enzymes, because of a superior understanding of the genetic organization of T7 and T3, were subsequently made from cloned genes and are now readily available. The logic of use of the RNA polymerases is identical for