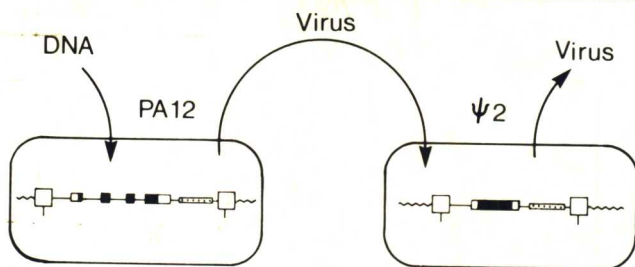
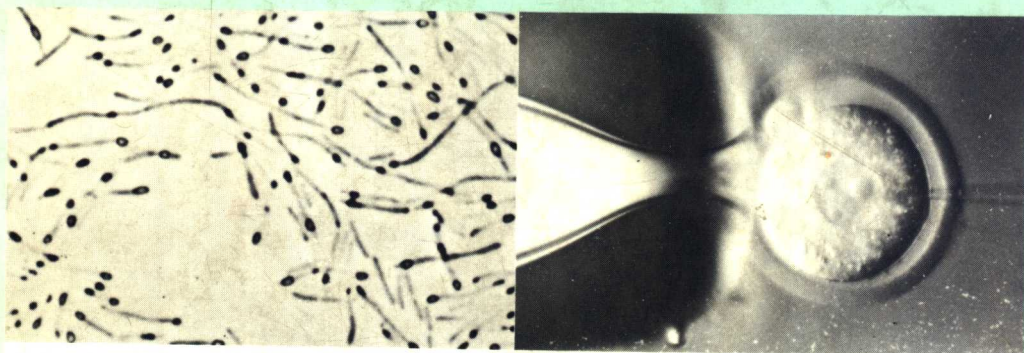


DNA cloning

Volume III

a practical approach

Edited by
D M Glover



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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 sulfoximine
ORF	open reading frame
PBS	phosphate-buffered saline
p.c.	post coitum
PDGF	platelet-derived growth factor
PEG	polyethylene glycol
PMSF	phenylmethylsulphonyl fluoride
RNAasin	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
Ylp	yeast integrating vectors

Contents

ABBREVIATIONS

xvii

1. APPLICATION OF PLASMIDS CONTAINING PROMOTERS SPECIFIC FOR PHAGE-ENCODED RNA POLYMERASES	1
Peter F.R.Little and Ian J.Jackson	
Scope of the Chapter	1
Bacteriophage Encoded RNA Polymerases	1
Background	1
Why use RNA probes?	2
Choice of vectors	3
Choice of RNA polymerase	5
Methods	6
Preparation of template DNA	6
Transcription reaction	7
Preparation of low specific activity or unlabelled RNA and alternative labels	10
Problems	10
The Use of RNA Probes	11
Southern hybridization	11
Northern hybridization	12
RNase protection	12
Specialist Procedures	16
In situ hybridization	16
Biological activity of in vitro transcribed RNA	16
Walking protocols	17
Sequencing RNA transcripts	17
Conclusion	17
Acknowledgements	18
References	18
 2. CHOICE AND USE OF COSMID VECTORS	 19
Peter F.R.Little	
Introduction	19
Scope of the chapter	19
Cosmid vectors	19
Choice of vectors	20
Cloning strategies	21
Methods	21
Preparation of insert DNA	21
Vector preparation	25
Ligation reactions	25

Packaging	26
Plating the library	26
Plating out	27
Screening cosmid filters	28
Storing cosmid libraries	30
Cosmid DNA preparations	33
Specialist Techniques	34
Cloning by recombination	34
Cos mapping	36
Walking	37
0.2% agarose gels	38
Problems	39
Starting DNA	39
Sucrose gradients	39
Failure of ligation or packaging	40
Plating cells	40
Plating out	40
Differential growth of cosmids	40
Vector problems	41
Conclusion	41
Acknowledgements	41
References	42

3. GENETIC APPROACHES TO THE CLONING, MODIFICATION AND CHARACTERIZATION OF COSMID CLONES AND CLONE LIBRARIES

43

Annemarie Poustka and Hans Lehrach

Introduction	43
Manipulation of Cosmid Clones by In Vivo Packaging	43
Selection of Cosmids by Homologous Recombination	45
Homologous recombination in E.coli can be used as alternative to colony hybridization	45
Principle of cosmid-plasmid recombination	46
Selection of recombinants	47
Vectors	47
Strains	48
Genetic selection of clones	49
Modification of clones	50
Protocols	52
Construction of cosmid libraries	52
In vivo packaging	54
Cosmid recombination	55
Acknowledgements	57
References	57

4. THE PURIFICATION OF EUKARYOTIC POLYPEPTIDES EXPRESSED IN ESCHERICHIA COLI	59
Fiona A.O.Marston	
Introduction	59
Expression vectors	59
Modes of expression	61
Cell Lysis Techniques	61
Total cell lysis	61
Preparation of spheroplasts	63
Insoluble Proteins	63
Isolation of inclusion bodies	63
Cleavage strategies for fusion proteins	65
Fusion proteins that facilitate purification	70
Solubilization of directly expressed proteins and fusion proteins	73
Refolding	75
Examples of eukaryotic polypeptides solubilized and refolded from E.coli	77
Soluble Proteins	82
Intracellular expression	82
Secretion	84
Concluding Remarks	85
Acknowledgements	86
References	86
Appendix	
Host vector combinations used for expression	87
 5. PRODUCTION AND PURIFICATION OF POLYCLONAL ANTIBODIES TO THE FOREIGN SEGMENT OF β-GALACTOSIDASE FUSION PROTEINS	 89
Sean B.Carroll and Allen Laughon	
Introduction—Expression Vectors for β -Galactosidase Fusion Proteins	89
λ gt11	90
pUR plasmid vectors	90
Construction and Screening of β -Galactosidase Fusions	91
Construction of λ gt11 fusions	92
Construction of pUR fusions	92
Screening of λ gt11 fusions	93
Screening of pUR fusions	95
Insert size versus yield	95
Verifying fusion protein integrity	95
Fusion Protein Expression and Purification	99
Induction of fusion proteins	99
Cell harvest and extraction of fusion proteins	100

Fusion protein purification — choosing a method	100
Polyclonal Antibody Production and Purification	105
Immunization schedules	105
Antiserum purification and assay	107
Acknowledgements	111
References	111
 6. PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST FUSION PROTEINS PRODUCED IN ESCHERICHIA COLI	 113
Sarah E.Mole and David P.Lane	
Introduction	113
Cloning and Expression of Gene Fragments	115
Construction of the fusion protein expressing vector	116
Derivation of Monoclonal Antibodies to Fusion Proteins	118
Preparation of the antigen	119
Immunization protocol	119
Detection of insert-specific antibodies	120
Setting up the hybridoma screen	122
The fusion	125
Freezing hybridoma cell lines	129
Cloning hybridoma cells	130
Contamination	130
Applications of Monoclonal Antibodies	131
Purification of monoclonal antibodies	132
Solid phase radioimmunoassays	132
Biotinylation	133
Epitope mapping	134
Further Applications: Shuttling of Insert-Containing EcoRI Cassettes	135
Examples	138
Acknowledgements	138
References	139
 7. EXPRESSION AND SECRETION OF FOREIGN GENES IN YEAST	 141
Bruce L.A.Carter, Meher Irani, Vivian L.MacKay, Ron L.Searle, Andrzej V.Sledziewski and Robert A.Smith	
Introduction	141
Methods included in this chapter	142
Expression vectors	142
Secretion vectors	146
Yeast Strains Suitable for Expression and their Construction	148

Maintenance of yeast stocks	150
Strain construction	150
Diploid construction	150
Yeast Transformation	153
Analysis of Transformants	156
Growth of Cells for Expression and Secretion Studies	158
Preparation of Samples for Analysis	159
Analysis of Yeast Expression	160
Purification of Yeast-Derived Recombinant Proteins	160
Acknowledgements	160
References	161

8. THE USE OF VECTORS BASED ON GENE AMPLIFICATION FOR THE EXPRESSION OF CLONED GENES IN MAMMALIAN CELLS 163

Christopher R.Bebbington and Christopher C.G.Hentschel

Introduction	163
Strategies for expression of cloned genes in mammalian cells	163
Gene amplification	164
Co-amplification and its uses	165
General features of amplifiable vectors	165
Methods described in this chapter	168
Protocols for Selection for Gene Amplification	168
Dihydrofolate reductase	168
Metallothionein	171
Glutamine synthetase	173
Adenosine deaminase	176
Planning a Strategy for Gene Amplification	179
Stability of gene amplification	180
Vector design	180
The frequency of gene amplification	181
Methods for Analysis of Gene Amplification	181
Measurement of gene copy number	181
Measurement of levels of RNA	182
Assays for protein expression levels	182
In situ hybridization to metaphase chromosomes	184
Acknowledgements	188
References	188

9. RETROVIRAL VECTORS 189

Anthony M.C.Brown and Michael R.D.Scott

Introduction	189
--------------	-----

Why retroviruses?	189
Applications of retroviral vectors	189
Retrovirus Biology	190
The retrovirus life-cycle	190
Structure of the retroviral genome and provirus	192
Receptors, tropism and immunity	193
Choosing a Vector	193
Principles of vector design	193
Replicative defective vectors	194
Replication component vectors	198
Preparation of insert sequences	198
Generation of Recombinant Virus Stocks	199
Production of helper-free stocks	199
Production of replication-competent virus stocks	202
Harvesting and storage of the virus; optimizing the titre	202
Infection of Target Cells	203
Infection with virus stocks	203
Infection by co-cultivation	203
Checking the Virus Stock	204
Titration of the virus	204
Checking the structure and expression of the recombinant virus	206
Recovering a provirus by cloning	206
Potential Problems	209
Inadequate dual expression	210
Genetic instability	211
Acknowledgements	211
References	211

10. THE PRODUCTION OF TRANSGENIC MICE BY THE MICROINJECTION OF CLONED DNA INTO FERTILIZED ONE-CELL EGGS	213
David Murphy and Jennifer Hanson	
Introduction	213
Transgenic animals	213
Routes to the germline	213
Animal Husbandry and Manipulation	217
Animal welfare and legal requirements	217
Animal and equipment requirements	218
Animal manipulations	220
Microinjection	234
Microinjection equipment	234
Microinjection of fertilized one-cell mouse eggs	237
DNA Manipulations	240
DNA for microinjection	240

Identification of transgenic mice	243
Transgenic Mice – Breeding and Gene Expression Analysis	245
Breeding transgenic lines	245
The expression of genes in transgenic mice	246
The application of transgenic technology	247
Acknowledgements	247
References	247
INDEX	249

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