

Contents

Chapter 1	Introduction	
1.1	The Beginning of Gene Cloning	1
1.2	How To Use This Book	3
1.3	What You Need To Know Before You Read This Book	5
1.4	A Request From the Authors	5
	Further Reading	6
Chapter 2	Genome Organization	
2.1	Introduction	7
2.2	The C-value Paradox	8
2.3	The Human Genome	9
2.4	Genomes of Other Eukaryotes	19
2.5	Bacterial Genomes	24
2.6	Plasmids	25
2.7	Viral Genomes	26
2.8	GC Content	27
2.9	Physical Characteristics of Eukaryotic Chromosomes	28
2.10	Karyotype	28
2.11	Euchromatin and Heterochromatin	30
2.12	CpG Islands	31
	Questions and Answers	32
	Further Reading	34
Chapter 3	Key Tools for Gene Cloning	
3.1	Introduction	35
3.2	Vectors	36
3.3	Restriction Enzymes	38
3.4	DNA Ligase	40
3.5	Transformation	42
3.6	Purification of Plasmid DNA	45
3.7	More Restriction Enzymes	47
3.8	Alkaline Phosphatase	51
3.9	More About Vectors	53
3.10	Analyzing Cloned DNA by Restriction Mapping	58
3.11	Measuring the Size of DNA Fragments	59
3.12	The Polymerase Chain Reaction and Its Use in Gene Cloning	64
3.13	How Does PCR Work?	67

3.14	Designing PCR Primers	72
3.15	The PCR Reaction	73
3.16	Uses for PCR Products	74
3.17	Cloning PCR Products	74
3.18	Real-time PCR for Quantification of DNA	76
3.19	Advantages and Limitations of PCR	76
	Questions and Answers	78
	Further Reading	83

Chapter 4 Gene Identification and DNA Libraries

4.1	The Problem	85
4.2	Genomic Library	87
4.3	Constructing a Genomic Library	87
4.4	How Many Clones?	89
4.5	Some DNA Fragments are Under-represented in Genomic Libraries	90
4.6	Using Partial Digests to Make a Genomic Library	90
4.7	Storage of Genomic Libraries	92
4.8	Advantages and Disadvantages of Genomic Libraries	92
4.9	Cloning Vectors for Gene Libraries	93
4.10	Vectors Derived from Bacteriophage λ	93
4.11	Packing Bacteriophage λ <i>In Vitro</i>	95
4.12	Cloning with Bacteriophage λ	97
4.13	Calculating the Titer of your Library	98
4.14	Cosmid Libraries	98
4.15	Making a Cosmid Library	99
4.16	YAC and BAC Vectors	100
4.17	cDNA Libraries	101
4.18	Making a cDNA Library	103
4.19	Cloning the cDNA Product	105
4.20	Expressed Sequence Tags	108
4.21	What are the Disadvantages of a cDNA Library?	108
	Questions and Answers	109
	Further Reading	116

Chapter 5 Screening DNA Libraries

5.1	The Problem	117
5.2	Screening Methods Based on Gene Expression	118
5.3	Complementation	119
5.4	Immunological Screening of Expression Libraries	120
5.5	Screening Methods Based on Detecting a DNA Sequence	123
5.6	Oligonucleotide Probes	124
5.7	Cloned DNA Fragments as Probes	127

	5.8 Colony and Plaque Hybridization	127
	5.9 Differential Screening	132
	5.10 Using PCR to Screen a Library	133
	Questions and Answers	135
	Further Reading	140
Chapter 6	Further Routes to Gene Identification	
	6.1 How Do We Get From Phenotype to Gene: a Fundamental Problem in Gene Cloning	141
	6.2 Gene Tagging: A Method That Both Mutates and Marks Genes	142
	6.3 A Simple Example of Transposon Tagging in Bacteria: Cloning Adherence Genes from <i>Pseudomonas</i>	149
	6.4 Signature-tagged Mutagenesis: Cloning Bacterial Genes with “Difficult” Phenotypes	152
	6.5 Gene Tagging in Higher Eukaryotes: Resistance Genes in Plants	156
	6.6 Positional Cloning: Using Maps to Track Down Genes	159
	6.7 Identification of a Linked Marker	161
	6.8 Moving From the Marker Towards the Gene of Interest	161
	6.9 Identifying the Gene of Interest	166
	6.10 Cloning of the CF Gene: A Case Study	168
	Questions and Answers	169
	Further Reading	171
Chapter 7	Sequencing DNA	
	7.1 Introduction	173
	7.2 Overview of Sequencing	174
	7.3 Sanger Sequencing	175
	7.4 The Sanger Sequencing Protocol Requires a Single-stranded DNA Template	179
	7.5 Modifications of the Original Sanger Protocol	181
	7.6 Strategies for Sequencing a DNA Fragment	182
	7.7 High-throughput Sequencing Protocols	184
	7.8 The Modern Sequencing Protocol	185
	7.9 Genome Sequencing	188
	7.10 High-throughput Pyrosequencing	197
	7.11 The Importance of DNA Sequencing	202
	Questions and Answers	203
	Further Reading	205
Chapter 8	Bioinformatics	
	8.1 Introduction	207
	8.2 What Does a Gene Look Like?	208

8.3	Identifying Eukaryotic Genes	214
8.4	Sequence Comparisons	217
8.5	Pair-wise Comparisons	217
8.6	Identity and Similarity	220
8.7	Is the Alignment Significant?	222
8.8	What Can Alignments Tell Us About the Biology of the Sequences Being Compared?	224
8.9	Similarity Searches	224
8.10	Fasta	226
8.11	BLAST	228
8.12	What Can Similarity Searches Tell Us About the Biology of the Sequences Being Compared?	230
8.13	Multiple Sequence Alignments	233
8.14	What Can Multiple Sequence Alignments Tell Us About the Structure and Function of Proteins?	234
8.15	Consensus Patterns and Sequence Motifs	235
8.16	Investigating the Three-dimensional Structures of Biological Molecules	237
8.17	Using Sequence Alignments to Create a Phylogenetic Tree	239
	Questions and Answers	242
	Further Reading	246
Chapter 9	Production of Proteins from Cloned Genes	
9.1	Why Express Proteins?	249
9.2	Requirements for Protein Production from Cloned Genes	252
9.3	The Use of <i>E. coli</i> as a Host Organism for Protein Production	252
9.4	Some Problems in Obtaining High Level Production of Proteins in <i>E. coli</i>	260
9.5	Beyond <i>E. coli</i> : Protein Expression in Eukaryotic Systems	265
9.6	A Final Word About Protein Purification	274
	Questions and Answers	275
	Further Reading	277
Chapter 10	Gene Cloning in the Functional Analysis of Proteins	
10.1	Introduction	279
10.2	Analyzing the Expression and Role of Unknown Genes	280
10.3	Determining the Cellular Location of Proteins	290
10.4	Mapping of Membrane Proteins	293
10.5	Detecting Interacting Proteins	297
10.6	Site-Directed Mutagenesis for Detailed Probing of Gene and Protein Function	304
	Questions and Answers	309
	Further Reading	312