

Contents

Acknowledgements	v
-----------------------------------	----------

Chapter 1. Introduction	1
--	----------

1.1. Preliminary remarks	1
1.2. Organisation of the book	4
1.3. General background to DNA sequencing	7
1.3.1. DNA synthesis with DNA polymerase I	8
1.3.2. Other properties of <i>E. coli</i> DNA polymerase I relevant to DNA sequencing	14
1.3.3. The Klenow sub-fragment of DNA polymerase I	15
1.3.4. Phage T ₄ -polymerase	15
1.3.5. Chain terminating inhibitors	16
1.3.6. Sequencing using end-labelled DNA	17
1.3.7. T ₄ -polynucleotide kinase	19
1.3.8. Restriction endonucleases	19
1.3.9. Properties of type II restriction endonucleases	20
1.3.10. Mapping DNA using restriction endonucleases	22
1.3.11. Cloning DNA sequences into plasmid and phage vectors	24
1.3.12. Cloning vehicles (vectors)	25
1.3.13. Insertion of DNA into a cloning vehicle	27
1.3.14. Conclusion	29

Chapter 2. DNA Sequencing	30
--	-----------

2.1. DNA sequencing by primed synthesis methods	30
2.1.1. Introduction	30
2.1.2. Principle of the 'plus and minus' method	31
2.2. Detailed protocol for the 'plus' and 'minus' primed synthesis method	40

2.3. Additional methods useful in conjunction with plus and minus method	46
2.3.1. Ribosubstitution method (Brown, 1978)	46
2.3.2. Experimental procedure	48
2.3.3. Discussion	50
2.3.4. Depurination analysis of defined fragments generated by primed synthesis	50
2.3.5. Detailed procedure	54
2.3.6. Buffers and mixes	61
2.4. Discussion and summary	61
2.5. Sequence analysis of short DNA fragments	64
2.5.1. Introduction	64
2.5.2. Procedures	65
2.5.3. Interpretation of results	68
2.5.4. Discussion	70

Chapter 3. Chain terminator sequencing 72

3.1. DNA sequencing with chain terminating inhibitors (Sanger et al., 1977)	72
3.1.1. Principle of the method	72
3.1.2. Detailed protocol and sequencing with chain-terminating inhibitors	73
3.1.3. Discussion	81
3.2. Sequencing by partial ribosubstitution	86
3.3. Direct enzymatic method for sequencing double-stranded restriction fragments using dideoxynucleoside triphosphates (Maat and Smith, 1978)	89
3.3.1. Introduction	89
3.3.2. Detailed protocol for nick-translation sequencing method	91
3.3.3. Discussion	93
3.3.4. A simplified dideoxynucleotide-nick translation sequencing procedure (Figure 3.11)	96
3.3.5. The forward-backward (F-B) procedure (Seif, Khoury and Dhar, 1980). Introduction	99
3.3.6. Experimental procedure	102
3.3.7. Discussion	103
3.4. The use of exonuclease III for preparing single-stranded DNA for use as a template in the chain terminator sequencing method (Smith, 1979, 1980)	104
3.4.1. Introduction	104
3.4.2. Experimental procedures	108
3.4.3. Results	113

<i>Chapter 4. Primed synthesis methods applied to DNA fragments cloned into phage M13</i>	<i>121</i>
4.1. General introduction	121
4.2. Rapid random sequencing by shotgun cloning into single stranded phage vectors (Sanger et al., 1980)	124
4.2.1. Preparation and properties of M13 cloning vectors and primers	124
4.2.2. Strategies for preparing DNA fragments for cloning into M13 vectors	138
4.2.2(a). End-labelling and addition of linkers	139
4.2.2(b). Removal of excess linkers and purification of products for cloning into the M13 vector	143
4.2.2(c). Random cleavage of DNA by methods other than using restriction endonucleases	145
4.2.3. Incorporation into vector DNA	149
4.2.4. Transformation	151
4.2.5. Preparation of single-stranded templates	152
4.2.6. Preparation of sequencing primer	153
4.2.7. Preliminary screening of templates with the ddT reaction (Sanger et al., 1980)	153
4.2.8. Sequencing the DNA	153
4.2.9. Gel electrophoresis	159
4.3. Detailed experimental procedures	161
4.3.1. Reagents	162
4.3.2. Solutions and media used for the cloning procedures	163
Experimental procedures	164
4.3.3. Preparation of starter culture of <i>E. coli</i> 71/18/JM101/JM103	164
4.3.4. Preparation of M13 RFI DNA	164
4.3.5. Linearization of vector DNA	166
4.3.6. Preparation of 'insert' DNA fragments	169
4.3.7. Preparation of the sequencing primers	175
4.3.8. Preparation and purification of restriction endonuclease fragments	178
4.3.9. Ligation into M13mp2 or M13mp7 vector DNA	182
4.3.10. Transformation	183
4.3.11. Preparation of single-stranded M13 DNA templates	185
4.4. Dideoxy-nucleotide sequencing procedures	187
4.4.1. Autoradiography	190
4.5. Results	190
4.6. Discussion	197
4.7. Hints for reading sequencing gels	200
4.8. Sequencing long single-stranded DNAs cloned in bacteriophage M13 using internal primers	206

4.8.1. Introduction	206
4.8.2. Principle of the method	207
4.8.3. Results	212
4.9. Sequencing cDNA from reverse transcripts of RNA	214
Key references	221

Chapter 5. DNA sequencing by the Maxam-Gilbert chemical procedure 230

5.1. Introduction	230
5.2. End-labelling DNA segments	236
5.3. Separating the two labelled ends of complementary strands	243
5.4. Strand separation	244
5.5. Elution of DNA from polyacrylamide gels	245
5.5.1. Elution by diffusion	245
5.5.2. Electroelution	245
5.6. Base-specific chemical cleavage reactions	248
5.6.1. Base modification reactions	250
5.6.2. Strand scission reaction	251
5.7. Gel electrophoresis	252
5.8. Reading the gel	253
5.9. Experimental procedures	255
5.10. Sequencing reactions (Maxam and Gilbert, 1980)	266
5.11. Trouble-shooting guide (Maxam and Gilbert, 1980)	270
5.12. Results	273
5.13. Strategies for sequencing double-stranded DNA	275

Update 286

Update to chapter 4	286
1. M13 sequencing primers	286
2. A system for making strand-specific M13 hybridization probes	287
3. A method for sequencing single stranded cloned DNA in both directions by the dideoxynucleotide-chain termination procedure	289
4. A systematic DNA sequencing strategy	290
5. Shotgun cloning into M13mp7	292
6. Effect of temperature on primed DNA synthesis in the presence of chain terminating inhibitors	293
7. Direct determination of sequences cloned into plasmid pBR233	294
Update to chapter 5	295

<i>Appendices</i>	297
Appendix 1. List of contractions and special terms	297
Appendix 2. Cloning vectors	300
Appendix 3. Commercially obtainable restriction endonucleases (1981)	301
Appendix 4. Autoradiography	302
Appendix 5. Equipment	304
Appendix 6.	307
Appendix 7. Nucleotide sequence of M13mp7; 7238 residues	307
Appendix 8. Restriction map of M13mp8 (7229 base pairs)	310
 <i>Chapter 6. Computer methods for DNA sequencers</i>	 311
1. Introduction	311
2. Data storage and retrieval: the DB system	318
3. Analysis: restriction sites, hairpin loops, translation, homologies	333
4. Choosing hardware and software	346
5. References	347
 <i>Appendix to chapter 6</i>	 349
Getting started with the DB system	349
Running the programs	350
 <i>References</i>	 369
<i>Subject index</i>	375