

CONTENTS

I. Definition	1
1. Structure and Expression of a Eukaryote Gene Coding for an mRNA and a Protein	3
2. Parameters of Description of a Genome	5
3. Sequencing of Whole Genomes	9
II. Vectors and Cloning	13
4. Restriction Enzymes	15
5. Electrophoresis of Nucleic Acids	19
6. Description of a Plasmid and a Phagemid	21
7. Description of a Bacteriophage and a Cosmid	23
8. Description of a YAC and Other Large Vectors	25
9. Molecular Cloning	27
10. Genetic Transformation of Bacteria and Yeasts	31
III. Labelling of Nucleic Acids and Hybridization	33
11. DNA Labelling	35
12. Molecular Hybridization	39
13. <i>In situ</i> Hybridization of mRNA	43
IV. DNA Libraries and Screening	47
14. Construction of a Genomic DNA Library	49
15. Construction of a cDNA Library	51
16. Screening of a Library	53
17. Differential Screening: Subtractive Libraries, AFLP-cDNA	57
18. Differential Display RT-PCR	61
19. Differential Screening by SSH: Suppression Subtractive Hybridization	65
20. Differential Screening by RDA: Representational Difference Analysis	69
21. EST: Expressed Sequence Tags	73
22. DNA Microarrays: DNA Microchips, cDNA Filters	77

V. Characterization of a Gene	89
23. DNA Sequencing	91
24. PCR: Polymerase Chain Reaction	97
25. RACE: Rapid Amplification of cDNA Ends	99
26. Genome Walking by PCR	103
27. RT-PCR: Reverse Transcriptase PCR	105
28. <i>In vitro</i> Transcription	109
29. Determination of Transcription Initiation Site	111
30. Functional Analysis of Promoters	113
31. Gel Retardation	117
32. DNase I Footprinting	119
VI. Genetic Transformation of Eukaryotes	121
33. Genetic Transformation of Plants by <i>Agrobacterium tumefaciens</i>	123
34. Direct Gene Transfer into Plant Protoplasts	129
35. Direct Gene Transfer by Biolistics	131
36. Genetic Transformation of Animal Cells	133
37. Cloning of Animals	137
38. Transient Expression	141
VII. Analysis of Gene Function	143
39. Recombinant Proteins	145
40. Baculoviruses of Insects, Vectors of Expression of Foreign Genes	149
41. Yeast two Hybrid System	155
42. Site-Directed Mutagenesis	161
43. Mutation Complementation in Yeast	167
44. Knock-out of Genes in Yeast	171
45. Gene Tagging	175
46. RNA Interference	181
VIII. Polymorphism of a Genome	185
47. Molecular Markers	187
48. Genetic and Physical Maps	193
49. PFGE: Pulse Field Gel Electrophoresis	197
50. RFLP: Restriction Fragment Length Polymorphism	201
51. RAPD: Random Amplified Polymorphic DNA	203
52. AFLP: Amplified Fragment Length Polymorphism	205
53. Retromarkers	207
54. SSCP: Single Strand Conformation Polymorphism	213

55. DGGE: Denaturing Gel Gradient Electrophoresis	215
56. SNP: Single Nucleotide Polymorphism	217
57. Simple Sequence Repeats (SSR): Microsatellites	219
List of Contributors	225