

Contents

Abbreviations	ix
Preface	xi

Chapter 1	An introduction to PCR	1
1.1	Introduction: PCR, a 'DNA photocopier'	1
1.2	PCR involves DNA synthesis	1
1.3	PCR is controlled by heating and cooling	3
1.4	PCR applications and gene cloning	5
1.5	History of PCR	6
Chapter 2	Understanding PCR	9
2.1	How does PCR work?	9
2.2	PCR: a molecular perspective	11
2.3	The kinetics of PCR	15
2.4	Getting started	18
2.5	Post-PCR analysis	18
	Protocol 2.1: Basic PCR	20
Chapter 3	Reagents and instrumentation	23
3.1	Technical advances in PCR	23
3.2	Reagents	23
3.3	PCR buffers	23
3.4	Nucleotides	25
3.5	Modified nucleotides	25
3.6	PCR premixes	26
3.7	Oligonucleotide primers	26
3.8	DNA polymerases for PCR	36
3.9	Early PCR experiments	37
3.10	Thermostable DNA polymerases	37
3.11	Properties of <i>Taq</i> DNA polymerase	37
3.12	Thermostable proofreading DNA polymerases	43
3.13	<i>Tth</i> DNA polymerase has reverse transcriptase activity	46
3.14	Red and green polymerases and reagents	47
3.15	Polymerase mixtures: high-fidelity, long-range and RT-PCRs	47
3.16	Nucleic acid templates	51
3.17	Mineral oil	54
3.18	Plasticware and disposables	54
3.19	Automation of PCR and thermal cyclers	55
	Protocol 3.1: Phosphorylation of the 5'-end of an oligonucleotide	63

Chapter 4	Optimization of PCR	65
4.1	Introduction	65
4.2	Improving specificity of PCR	65
4.3	Template DNA preparation and inhibitors of PCR	75
4.4	Nested PCR improves PCR sensitivity	76
4.5	Contamination problems	76
4.6	Preventing contamination	80
4.7	Troubleshooting guide	82
Chapter 5	Analysis, sequencing and <i>in vitro</i> expression of PCR products	87
5.1	Introduction	87
5.2	Analysis of PCR products	87
5.3	Verification of initial amplification product	89
5.4	Direct DNA sequencing of PCR products	93
5.5	Direct labeling of PCR products and homogenous assays	101
5.6	<i>In vitro</i> expression of PCR products	103
	Protocol 5.1: Cycle sequencing – Applied Biosystems Big Dye terminators	108
Chapter 6	Purification and cloning of PCR products	111
6.1	Introduction	111
6.2	Purification of PCR products	111
6.3	Introduction to cloning of PCR products	115
6.4	Approaches to cloning PCR products	117
6.5	Confirmation of cloned PCR fragments	131
	Protocol 6.1: Blunt-end polishing of PCR fragments	134
	Protocol 6.2: PCR screening of bacterial colonies or cultures	135
Chapter 7	PCR mutagenesis	137
7.1	Introduction	137
7.2	Inverse PCR mutagenesis	138
7.3	Unique sites elimination	144
7.4	Splicing by overlap extension (SOEing)	144
7.5	Point mutations	150
7.6	Deletions and insertions	151
7.7	Deletion mutagenesis	151
7.8	Insertion mutagenesis	151
7.9	Random mutagenesis	157
7.10	PCR misincorporation procedures	159
7.11	Recombination strategies	160
7.12	RACHITT	166
7.13	Gene synthesis	166
	Protocol 7.1: Inverse PCR mutagenesis	171
	Protocol 7.2: Quikchange mutagenesis of plasmid DNA	173
	Protocol 7.3: Splicing by Overlap Extension (SOEing)	175
	Protocol 7.4: 'Sticky-feet' mutagenesis	177

	Protocol 7.5: DNA shuffling	179
	Protocol 7.6: Gene synthesis	182
Chapter 8	Analysis of gene expression	185
	8.1 Introduction	185
	8.2 Reverse transcriptase PCR (RT-PCR)	185
	8.3 Semi-quantitative and quantitative RT-PCR	189
	8.4 One-tube RT-PCR	194
	8.5 Differential display	194
	8.6 PCR in a cell: <i>in situ</i> RT-PCR	198
	8.7 Microarrays	204
	8.8 RNA interference (RNAi)	205
	Protocol 8.1: Reverse transcriptase reaction	208
Chapter 9	Real-time RT-PCR	209
	9.1 Introduction	209
	9.2 Basic principles of real-time RT-PCR	209
	9.3 Detection methods	212
	9.4 General guidelines for probe and primer design	221
	9.5 Instruments and quantification of results	222
	9.6 Normalization and control selection	225
	9.7 A typical real-time RT-PCR experiment using SYBR® Green I	225
	9.8 Common real-time RT-PCR pitfalls	228
	9.9 Applications of real-time RT-PCR	229
Chapter 10	Cloning genes by PCR	233
	A Cloning genes of known DNA sequence	233
	10.1 Using PCR to clone expressed genes	233
	10.2 Express sequence tags (EST) as cloning tools	237
	10.3 Rapid amplification of cDNA ends (RACE)	238
	B Isolation of unknown DNA sequences	240
	10.4 Inverse polymerase chain reaction (IPCR)	240
	10.5 Multiplex restriction site PCR (mrPCR)	243
	10.6 Vectorette and splinkerette PCR	244
	10.7 Degenerate primers based on peptide sequence	248
	Protocol 10.1: 5'-RACE	253
	Protocol 10.2: Inverse PCR from plant genomic DNA	255
Chapter 11	Genome analysis	257
	11.1 Introduction	257
	11.2 Why map genomes?	258
	11.3 Single-strand conformation polymorphism analysis (SSCP)	259
	11.4 Denaturing-high-performance liquid chromatography (DHPLC)	263
	11.5 Ligase chain reaction (LCR)	264
	11.6 Amplification refractory mutation system (ARMS)	264

11.7	Cleaved amplified polymorphic sequence analysis (CAPS)	267
11.8	SNP genotyping using DOP-PCR	268
11.9	Random amplified polymorphic DNA (RAPD) PCR	269
11.10	Amplified fragment length polymorphisms (AFLPs)	270
11.11	Multiplex PCR analysis of <i>Alu</i> polymorphisms	270
11.12	Variable number tandem repeats in identity testing	271
11.13	Minisatellite repeat analysis	274
11.14	Microsatellites	276
11.15	Sensitive PCR for environmental and diagnostic applications	277
11.16	Screening transgenics	278

Index**283**