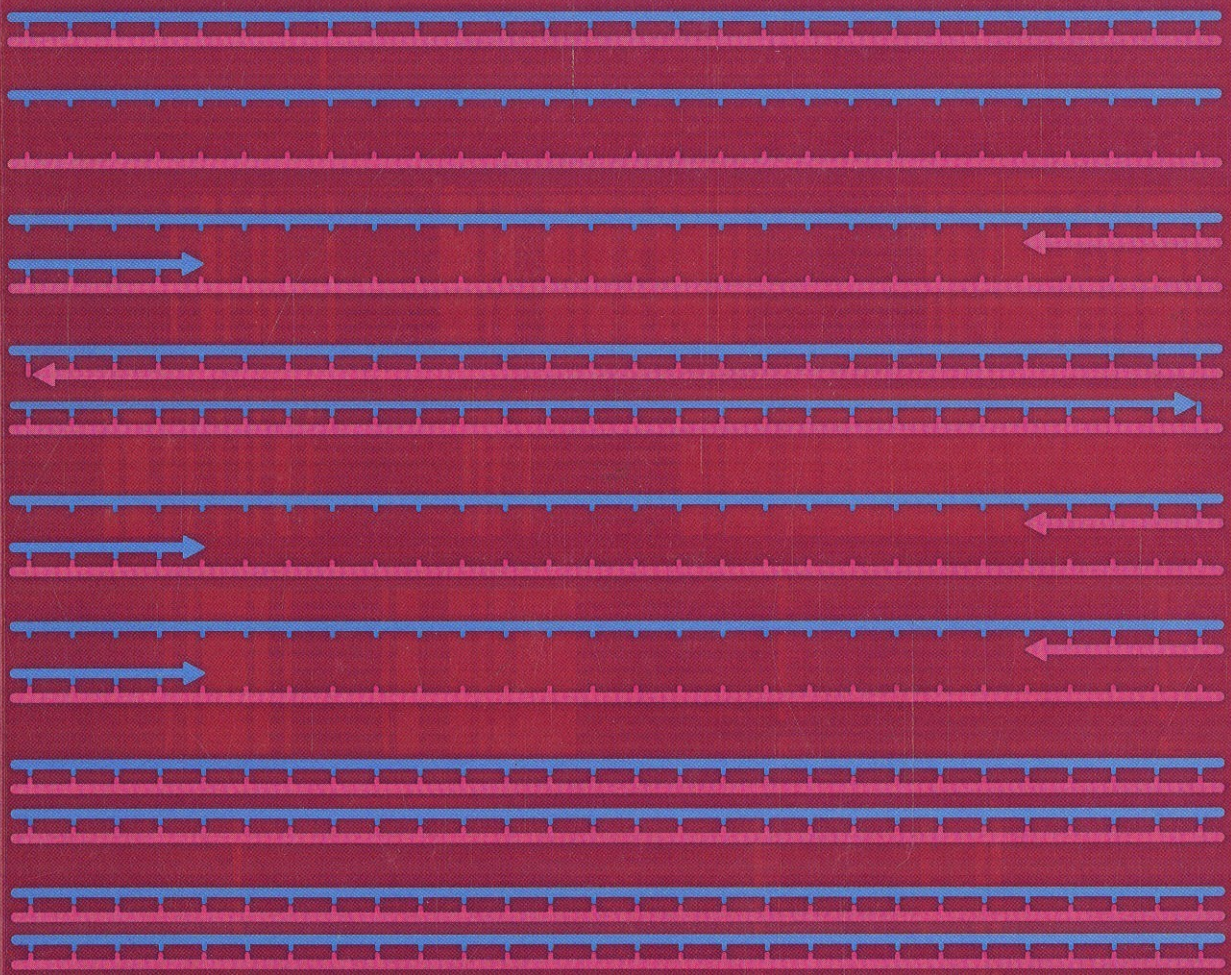
METHODS EXPRESS

PCR

edited by S. Hughes and A. Moody



Contents

Contributors xiii
Foreword xvii
Preface xviii
Abbreviations xix

Color section xxi

Chapter 1.
An introduction to the polymerase chain reaction
Adrian Moody
1. Introduction 1
    1.1 History of PCR 1
    1.2 Utility of PCR – modifications and applications 3
2. Methods and approaches 4
    2.1 Components of PCR 4
    2.2 The PCR cycle 5
    2.3 Thermal cyclers 6
    2.4 Oligonucleotide primers 6
    2.5 Standard PCR 8
    2.6 Optimizing a PCR 11
    2.7 Optimizing $MgCl_2$ concentration 13
    2.8 General hints and tips 17
3. Troubleshooting 18
4. References 20

Chapter 2.
Polymerases for PCR
Meg Martel, Simon Baker, Ian Kavanagh, and Simon May
1. Introduction 21
    1.1 Function of DNA polymerases 21
    1.2 Origins of DNA polymerases 21

2. Methods and approaches 22
2.1 Unit definition of DNA polymerase 22
2.2 DNA polymerase activity 22
2.3 Choice of enzyme 22
2.4 Hot-start PCR 24
2.5 Buffer composition 26
2.6 Inhibitors 27
2.7 Detection methods for assessing enzyme activity 27
3. Troubleshooting 33
4. References 33

Chapter 3.
A detailed guide to quantitative RT-PCR
Pete Kaiser
1. Introduction 35
2. Methods and approaches 36
2.1 Technologies available 36
2.2 Choice of reference gene 38
2.3 Analysis of real-time qRT-PCR data 38
2.4 Designing TaqMan primers and probes 41
2.5 Recommended protocols 41
2.6 Analyzing primer optimization results 43
3. Troubleshooting 46
4. References 47

Chapter 4.
Use of quantitative PCR for the detection of genomic microdeletions or microduplications
Simon Hughes, Rosanna Weksberg, Laura Moldovan, and Jeremy A. Squire
1. Introduction 49
1.1 Test model 50
2. Methods and approaches 51
2.1 SYBR Green 51
2.2 Measuring amplification 51
2.3 Primer design 53
2.4 Housekeeping/reference genes and data normalization 54
2.5 Primer optimization 54
2.6 Generating a standard curve 54
2.7 Recommended protocols 56
2.8 DNA quantification and data analysis 58
2.9 Case study 58
2.10 Concluding remarks 60
3. Troubleshooting 61
4. References 62

Chapter 5.
Robust and unique PCR for single-nucleotide polymorphism genotyping applications
Xiangning Chen
1. Introduction 63
   1.1 Variable number of tandem repeats 63
   1.2 Single-nucleotide polymorphisms 64
2. Methods and approaches 65
   2.1 General guidelines for genotyping applications 65
   2.2 SNP genotyping methods 68
   2.3 General considerations 70
   2.4 Recommended protocols 70
   2.5 Typical results 75
   2.6 Summary 76
3. Troubleshooting 77
4. References 79

Chapter 6.
Using PCR and linkage mapping to identify single genes and quantitative trait loci for livestock traits
Jillian F. Maddox, Imke Tammen, and Sonja Dominik
1. Introduction 81
   1.1 Genetic traits 81
   1.2 Genetic markers 82
2. Methods and approaches 83
   2.1 Genotyping 83
   2.2 Data analysis: linkage analysis and trait mapping 100
   2.3 Definition of terms 102
3. Troubleshooting 103
4. References 104

Chapter 7.
PCR restriction fragment length polymorphism analysis for genotyping of single-nucleotide polymorphisms
Simon Hughes
1. Introduction 107
   1.1 Restriction enzymes 107
   1.2 RFLP 108
   1.3 PCR-RFLP 108
2. Methods and approaches 109
   2.1 Restriction enzyme identification 110
   2.2 DNA extraction and PCR amplification 116
   2.3 Results and data interpretation 119
3. Troubleshooting 119
4. References 121

Chapter 8.
Forensic genetic DNA typing with PCR-based methods
Claus Børsting, Juan J. Sanchez, and Niels Morling
1. Introduction 123
   1.1 PCR and forensic genetics 123
   1.2 Mitochondrial DNA analysis 124
   1.3 Single nucleotide polymorphisms 125
2. Methods and approaches 125
   2.1 PCR fragment analysis 125
   2.2 PCR optimization 125
   2.3 PCR multiplexing 126
   2.4 Recommended protocols 127
3. Troubleshooting 140
4. References 141

Chapter 9.
Large PCR multiplexes with special reference to forensic single-nucleotide polymorphism typing
Juan J. Sanchez, Claus Børsting, and Niels Morling
1. Introduction 143
   1.1 Genetic markers 143
   1.2 SNP typing 144
   1.3 SNP multiplexing 145
2. Methods and approaches 146
   2.1 Multiplex design 146
   2.2 Recommended protocols 148
   2.3 Multiple-injection protocol 153
3. Troubleshooting 156
4. References 157

Chapter 10.
Pre-implantation genetic diagnosis of monogenic disease: PCR-based methods for the identification of mutations in single cells
Dagan Wells
1. Introduction 159
   1.1 Principles of PGD 159
   1.2 Contamination 160
   1.3 Amplification efficiency 162
   1.4 Allele dropout 163
   1.5 Whole genome amplification 163
2. Methods and approaches 165
   2.1 Expected results from multiplex PCR 169
3. Troubleshooting 170
4. References 171

Chapter 11.
Rapid generation of gene-targeting constructs
Trevor J. Wilson, Dirk Truman, Antonietta Giudice, and Paul Hertzog
1. Introduction 173
   1.1 The function (or phenotype)-driven approach 173
   1.2 The gene-driven approach 174
2. Methods and approaches 176
   2.1 Obtaining gene sequences and identification of BACs 178
   2.2 ET cloning procedure 182
   2.3 Cloning of floxed exon 185
   2.4 Preparation and electroporation of DNA 186
   2.5 Screening of ES cell clones 195
3. Troubleshooting 195
4. References 196

Chapter 12.
Construction of long DNA molecules from multiple fragments using PCR
Nikolai A. Shevchuk and Anton V. Bryksin
1. Introduction 197
2. Methods and approaches 197
   2.1 Principles of long multiple fusion 197
   2.2 Limitations of long multiple fusion 199
   2.3 Factors critical for successful long multiple fusion 200
   2.4 Recommended protocols 203
3. Troubleshooting 214
4. References 214

Chapter 13.
Efficient PCR-based mutagenesis method applicable to diverse mutagenesis strategies using type IIs restriction enzymes
Jae-Kyun Ko and Jianjie Ma
1. Introduction 217
2. Methods and approaches 218
   2.1 Principles of mutagenesis 218
   2.2 Design of mutagenic primers and choice of type IIs restriction enzyme for mutagenesis 219
   2.3 Application to diverse mutagenesis 224
   2.4 Summary 225
3. Troubleshooting 225
4. References 228

Chapter 14.
Inverse PCR-based restriction fragment length polymorphism for identifying low-level mutations in tumors
G. Mike Makrigiorgos
1. Introduction 229

2. Methods and approaches 230
2.1 Principles of inverse PCR-based amplified restriction fragment length polymorphism 230
2.2 Size-separation analysis of PCR products and estimation of mutation frequencies 236
2.3 Sample results 236
3. *Troubleshooting* 240
4. References 240

Chapter 15.
PCR methods for infectious disease diagnosis
Padmini Ramachandran, Andrew Hardick, Charlotte Gaydos, Samuel Yang, and Richard Rothman
1. Introduction 243
1.1 PCR for infectious disease diagnosis in a clinical setting 244
1.2 Antimicrobial resistance profiling 245
1.3 PCR and biodefense 245
2. *Methods and approaches* 245
2.1 Cost of PCR 245
2.2 False-negative and -positive results 246
2.3 Sample processing for PCR 247
2.4 Sample preparation from various human specimens 247
2.5 Recommended protocols 249
3. Troubleshooting 261
4. References 263

Chapter 16.
Use of PCR for DNA methylation analyses
Mario F. Fraga and Manel Esteller
1. Introduction 265
2. Methods and approaches 266
2.1 PCR-based techniques 266
2.2 Summary 275
3. Troubleshooting 275
4. References 277

Chapter 17.
PCR-based methods to determine DNA methylation status at specific CpG sites using methylation-sensitive restriction enzymes
Helmtrud I. Roach and Ko Hashimoto
1. Introduction 279
2. Methods and approaches 280
2.1 Methylation-sensitive restriction enzymes 280
2.2 Principle of the MSRE PCR method 281
2.3 Identifying CpG sites and suitable MSREs 282
2.4 Extraction of nucleic acids 283

2.5 Detection of methylation status using MSREs 286
2.6 Applications 289
3. Troubleshooting 290
4. References 291

Chapter 18.
PCR-based whole genome amplification
Nona Arneson, Simon Hughes, Richard Houlston, and Susan Done
1. Introduction 293
2. Methods and approaches 294
2.1 DOP-PCR 296
2.2 PEP-PCR 299
2.3 Ligation-mediated PCR 301
2.4 Downstream applications 313
3. Troubleshooting 314
3.1 General troubleshooting 314
3.2 I-PEP-PCR 316
3.3 PRSG 316
4. References 316

Chapter 19.
PCR *sequencing of human genes for the discovery of DNA sequence variants*
Abizar Lakdawalla
1. Introduction 319
1.1 DNA sequence variations 319
2. Methods and approaches 320
2.1 Resequencing methods 320
2.2 Analysis of results 334
2.3 Mutation nomenclature 339
3. Troubleshooting 340
4. References 342

Appendix 1
List of suppliers 343

Index 345