

Contents

Author Biographies

ix

1. Things to Consider Before Preparing to Undertake Your First Polymerase Chain Reaction	
1.1 Introduction	1
1.2 Basic Laboratory Preparation	2
1.3 Maintaining a Nuclease-Free Environment	4
1.4 Consideration of Timescales	5
1.5 General Protocol for Extracting DNA	6
1.6 What Reagents You Will Need and Where to Buy Them	8
1.7 Safety Considerations	9
Summary	9
2. Designing and Ordering Your Polymerase Chain Reaction Primers	
2.1 Introduction	11
2.2 Choosing a "Template"	12
2.3 Finding Gene Sequences Using Online Databases	13
2.4 Identifying Your Amplicon of Interest	23
2.5 Contending With Introns and Exons	23
2.6 Basic Primer Parameters	23
2.7 Calculating Annealing and Melting Temperatures	26
2.8 Tools for Primer Design	26
2.9 Ordering Your Primers	27
2.10 Reconstituting Primers and Preparing a Working Stock	28
2.11 Testing a New Primer Pair	28
2.12 Common Problems and Troubleshooting	30
3. Analyzing Your Template DNA and/or PCR Product	
3.1 Introduction	31
3.2 Basic DNA/PCR Analysis	31
3.3 "Running" an Agarose Gel	32

3.4	Staining and Visualizing DNA	37
3.5	Which Molecular Weight Marker is Best for You?	38
3.6	Imaging Your Gel and Interpreting What It Shows	39
3.7	Quantifying DNA/PCR Product Using Electrophoresis	41
	Example Calculation	43
	Summary	43
4.	Quantitative PCR: Things to Consider	
4.1	Introduction	45
4.2	What are You Trying to Achieve?	45
4.3	The Importance of Housekeeping	46
4.4	What Q-PCR Machine Should You Use, or What Machine Is Available to You?	47
4.5	Efficiency Parameters	47
4.6	Linearity and Amplification Efficiency Based in the Template Material	49
4.7	Amplification Efficiency	50
4.8	cDNA Conversion and Quantification	51
4.9	Planning to Use Q-PCR for Multiplex	52
	Summary	52
5.	Carrying Out Q-PCR	
5.1	Introduction	53
5.2	Reagents	53
5.3	Basic Protocols	55
5.4	Absolute Quantification	56
5.5	Relative Quantification	57
	Summary	59
6.	Using PCR for Cloning and Protein Expression	
6.1	Introduction	61
6.2	Enzymes for Cloning	61
6.3	"Cleaning" PCR Products for Cloning	62
6.4	Directional Cloning	63
6.5	Ligation of PCR Product and Vector	65
6.6	Transforming and Analyzing the Plasmid Construct	66
6.7	Sequencing Plasmid Constructs	70
	Summary	71
7.	Polymerase Chain Reaction for Knocking Out Genes	
7.1	Introduction	73
7.2	How to Knockout a Gene Using PCR	74

7.3	Transferring Engineered PCR Fragments into the Target Organism Using a Suicide Vector	76
7.4	The λ Red Recombinase System	77
7.5	Screening for Knockout Mutations	78
	Summary	79
Appendix 1: Trouble Shooting		81
Appendix 2: Suppliers		83
Index		85