Contents

Preface		xi
About the Autho	ors	xiii
Acknowledgeme	ents	XV
Note to Instruct	ors	xvii
Instrumentatio	n	xix
Nomenclature		xxi
Experimenta Laboratory S	Outline for Experiments al Procedures Safety trating Procedures	xxiii xxiii xxiii xxv xxvi
PART 1 – Manip	pulation of DNA	; 1
Lab Session 1	Reference Getting Oriented: Practicing with Micropipettes	2 3
	Station Checklist Micropipetting Micropipetting Self-Test Laboratory Exercise: BSA Serial Dilutions and Nitrocellulose Spot Test Preparing BSA Dilutions Performing a Nitrocellulose Spot Test Discussion Questions	3 4 6 7 7 7 9
Lab Session 2	Purification and Digestion of Plasmid (Vector) DNA	11
	Introduction to Plasmid Purification Alkaline Lysis Silica Adsorption DNA Quantification Introduction to Expression Vectors Principles of Gene Expression Expression Vectors	11 11 12 12 12 13

	Orientation and Reading Frame Orientation Reading Frame Laboratory Exercises Alkaline Lysis and Silica Adsorption Protocol DNA Quantification Restriction Digestion of Expression Vector DNA pET-41a, a GST Fusion Protein Vector Discussion Questions Reference	14 14 15 16 16 18 19 20 20	
Lab Session 3	PCR Amplification of egfp and Completion of Vector Preparation	21	
	Introduction What is the Polymerase Chain Reaction (PCR)? Why Clone by PCR? TA Cloning PCR Cloning by Incorporation of Restriction Sites Cloning Synthetic Genes Laboratory Exercises PCR Amplification of egfp from the pEGFP-N1 Plasmid PCR Protocol Clean-up of Digested pET-41a Vector Agarose Gel Electrophoresis Discussion Questions References	21 23 23 23 24 25 25 25 27 29	
Lab Session 4	Preparation of Insert DNA (egfp) PCR Product	31	
	Check PCR Reactions on an Agarose Gel Spin Column Cleanup of PCR Product Quantification of egfp PCR Product Restriction Digestion of egfp PCR Product Removing Enzymes and Cleaning Digested DNA Using a Spin Column Discussion Questions	31 31 32 32 33	
Lab Session 5	DNA Ligation and Transformation of Escherichia coli	35	
	Introduction Ligation Transformation Laboratory Exercises Ligations and Ligation Controls Divalent Cation-Mediated Transformation Electrophoresis of Ligation Reactions Discussion Questions Reference	35 36 37 37 39 39 40 40	
PART 2 – Screening Transformants 41			
Lab Session 6	Colony Hybridization	43	

Lab Session 6A	Interim Laboratory Session	44
	Introduction Laboratory Exercises	44 44
	Counting Transformants and Replica Plating Replica Plating	44 44
Lab Session 6B	Colony Hybridization: Monoclonal Antibody Probe	46
	Introduction Laboratory Exercises Colony Hybridization with an α -EGFP Monoclonal Antibody Probe: Part 1	46 47 47
Lab Session 6	Discussion Questions	49
Lab Session 7	Characterization of Recombinant Clones: Part 1	51
Lab Session 7A	Completion of Colony Hybridization with a Monoclonal Antibody Probe	52
	Introduction	52
	Laboratory Exercise Colony Hybridization with an α -EGFP Monoclonal Antibody Probe: Part 2	52 52
Lab Session 7B	PCR Screening	54
	Introduction	54
	Laboratory Exercise	54
	Polymerase Chain Reaction Screen for Recombinant Clones	54
Lab Session 7C	Prepare Fresh Replica Plate	57
Lab Session 7	Discussion Questions	57
Lab Session 8	Characterization of Recombinant Clones: Part 2	59
Lab Session 8A	Interim Laboratory Session	60
	Laboratory Exercise Inoculate Cultures for Minipreps	60 60
Lab Session 8B	Analysis of PCR Screen Results	61
	Introduction Laboratory Exercise	61 61
	Gel Electrophoresis and Analysis of PCR Samples from Last Week	61
Lab Session 8C	Isolation of Miniprep DNA from Potential Transformants	62
	Introduction	62
	Laboratory Exercise	63
	Isolation of Miniprep DNA from Potential Transformants Plasmid DNA Purification Using the QIAprep Spin Miniprep Kit	63
	and a Microcentrifuge	63

Lab Session 8D	Visualization of Green Fluorescent Protein: Part 1	65
	Introduction	65
	Laboratory Exercise	65
	Green Fluorescence Assay and Preparation of a Fresh Master Plate	65
Lab Session 8	Discussion Questions	66
Lab Session 9	Characterization of Recombinant Clones: Part 3	67
Lab Session 9A	Characterization of Miniprep DNA from Potential Transformants (Restriction Enzyme Analysis of Putative Transformants)	68
	Introduction	68
	Laboratory Exercise	68
	Restriction Enzyme Analysis of Miniprep DNA	68
Lab Session 9B	Visualization of Green Fluorescent Protein: Part 2	70
	Introduction	70
	Laboratory Exercise Visualization of Clones Expressing the Enhanced Green Fluorescent Protein	70
	on IPTG Plates	70
Lab Session 9C	Computational Analysis of DNA Sequence from a Positive Clone: Part 1	72
	Introduction	72
	Laboratory Exercise	74
	References	76
Lab Session 9	Discussion Questions	76
Lab Session 10	Computational Analysis of DNA Sequence from a Positive Clone: Part 2	77
	Introduction	77
	Laboratory Exercise Discussion Questions	81
	Reference	85
		0.
-	ssion, Detection and Purification of Recombinant Proteins from Bacteria	87
Lab Session 11	Expression of Fusion Protein from Positive Clones, SDS-PAGE and Western Blot: Part 1	89
Lab Session 11A	Interim Laboratory Session	90
	Laboratory Exercise	90
	Inoculate Cultures for SDS-PAGE	90
Lab Session 11B	Expression of Fusion Protein from Positive Clones, SDS-PAGE and Western Blot	91
	Introduction Laboratory Exercise	91 93
	SDS-PAGE and Western Blot: Part 1	93
	Reference	97
Lab Session 11	Discussion Questions	97

Contents

Lab Session 12	Expression of Fusion Protein from Positive Clones, SDS-PAGE and Western Blot: Part 2	99
	Introduction	99
	Laboratory Exercises	99
	SDS-PAGE and Western Blot: Part 2	99
	Replica Plate Positive Clone	101
	Discussion Questions	101
Lab Session 13	Extraction of Recombinant Protein from <i>Escherichia coli</i> Using a	102
	Glutathione Affinity Column	103
Lab Session 13A	Interim Laboratory Session	104
	Laboratory Exercise	104
	Inoculate Cultures for Protein Purification	104
Lab Session 13B	Extraction of Recombinant Protein from Escherichia coli and Purification Using a	
	Glutathione Affinity Column	105
	Introduction	105
	Laboratory Exercises	108
	Growing Bacterial Suspension Cultures for Fusion Protein Purification	108
	Harvesting IPTG-Induced Cultures	108
	Breaking Open Bacterial Cells	108
	Removing Insoluble Debris from the Crude Homogenate	109
	Purifying Protein by Affinity Chromatography	109
Lab Session 13	Discussion Questions	111
Lab Session 14	Analysis of Purification Fractions	113
Lab Session 14A	Analysis of Purification Fractions	114
	Introduction	114
	Laboratory Exercises	115
	SDS-PAGE of Purified Fusion Protein	115
	Fluorescence Analysis of Affinity Purification	116
Lab Session 14B	Replica Plate Positive Clone	120
Lab Session 14	Discussion Questions	120
DART 4 _ Analys	sis of mRNA Levels	121
rant 4-Analys	Challenges of Working with RNA	122
	References	123
Lab Session 15	Total RNA Purification	125
Lab Session 15A	Interim Laboratory Session	126
	Laboratory Exercise	126
	Inoculate Cultures for RNA Purification	126

Lab Session 15E	3 Total RNA Purification	127
	Introduction Laboratory Exercises Purification of Total RNA DNase Digestion Quantification of RNA References	127 129 129 131 131
Lab Session 15	Discussion Questions	132
Lab Session 16	Analysis of gst::egfp mRNA Levels by RT-qPCR: Part 1	133
	Introduction Reverse Transcription Quantitative PCR Laboratory Exercises Reverse Transcription Quantitative PCR (qPCR) Discussion Questions Reference	133 134 135 137 137 138 140
Lab Session 17	Analysis of gst::egfp mRNA Levels by RT-qPCR: Part 2	141
Lab Session 18	Introduction Laboratory Exercise Relative Quantification of gst::egfp Levels References Discussion Questions Analysis of gst::egfp mRNA Levels by Semi-Quantitative RT-PCR: Part 1	141 144 144 146 146
Lab Session 10	Introduction Laboratory Exercises Reverse Transcription (RT) Semi-Quantitative PCR Discussion Questions	147 148 148 148 148
Lab Session 19	Analysis of gst::egfp mRNA Levels by Semi-Quantitative RT-PCR: Part 2	153
Appendix 1: Eqi	Introduction Laboratory Exercises Agarose Gel Electrophoresis Quantification Discussion Questions Reference	153 153 153 154 155 155
Appendix 2: Pre		159
Appendix 3: Pre Appendix 4: Pre	paration of Competent E. coli Cells -Lab Questions	181 185
Index		197