

Contents

Preface	xiii
Acknowledgments	xv
Suggested Schedule for Exercises	xvii

Introductory Notes

Record Keeping and Safety Rules	
Format of Student Laboratory Records	3
Ten Dos and Don'ts of Record Keeping	5
Criteria for Grading the Laboratory Notebook	7
Safety Rules in the Laboratory	9

Exercise 1

Measurement of pH	11
-------------------	----

Exercise 2

Use of Micropipettors and Spectrophotometers	17
--	----

Exercise 3

Aseptic Technique: Transferring a Culture	23
---	----

Exercise 4

Establishing a Pure Culture: The Streak Plate 27

Exercise 5

Preparation of Culture Media 33

Exercise 6

The Growth Curve 41

Exercise 7Isolation of Plasmid DNA from *Escherichia coli*:
The Mini-Prep 47**Exercise 8**Purification, Concentration, and
Quantitation of DNA 53**Exercise 9**

Isolation of Plasmid DNA: The Maxi-Prep 61

Exercise 10Restriction Digestion and Agarose
Gel Electrophoresis 71**Exercise 11**

Southern Transfer 81

Exercise 12Preparation, Purification, and
Hybridization of Probe 91**Exercise 13**Transformation of *Saccharomyces cerevisiae* 103

Exercise 14

Transformation of *Escherichia coli* by
Plasmid DNA 109

Exercise 15

Protein Assays 115

Exercise 16

β -Galactosidase Assay 121

Exercise 17

Determination of β -Galactosidase in
Permeabilized Yeast Cells 125

Exercise 18

Assay of β -Galactosidase in Cell Extracts 129

Exercise 19

β -Galactosidase Purification 135

Part A Gel Filtration Chromatography:
Column Calibration 136

Part B Ammonium Sulfate Salting-Out of β -Galactosidase and
Column Chromatography 140

Part C Sodium Dodecyl Sulfate–Polyacrylamide
Gel Electrophoresis 144

Appendix 1

Alternative Protocols and Experiments

Exercise 6A Isolation and Characterization of Auxotrophic
Yeast Mutants 153

Exercise 9A Large-Scale Isolation of Plasmid DNA by
Column Chromatography 158

Exercise 12A Colony Hybridization 164

Appendix 2

Buffer Solutions 167

Appendix 3

Preparation of Buffers and Solutions 170

Appendix 4Properties of Some Common Concentrated
Acids and Bases 174**Appendix 5**

Use of Micropipettors 175

Appendix 6

Safe Handling of Microorganisms 178

Appendix 7

List of Cultures 181

Appendix 8

Storage of Cultures 182

Appendix 9

Sterilization Methods 184

Appendix 10

Preparation of Stock Solutions for Culture Media 186

Appendix 11

Growth in Liquid Medium 188

Appendix 12

Determination of Viable Cells 191

Appendix 13

Determination of Cell Mass 193

Appendix 14

Determination of Cell Number 195

Appendix 15

Nomenclature of Strains 198

Appendix 16

Glassware and Plasticware 201

Appendix 17

Preparation of Tris and EDTA 203

Appendix 18

Basic Rules for Handling Enzymes 206

Appendix 19

Manufacturers' and Distributors' Addresses 210

Glossary 217

Index 223