

Short Contents

Introduction xi

EXPERIMENTS

1. Plasmid Purification and Restriction Mapping 1
2. Affinity Purification of Histidine-Tagged FnbA Protein 29
3. Polymerase Chain Reaction and DNA Sequence Analysis of Bacterial Ribosomal RNA Genes 43
4. Terminal Restriction Fragment Length Polymorphism Analysis of 16S Ribosomal RNA Genes from Uncultured Bacterial Communities 71

APPENDICES

- A. Sample Laboratory Reports 103
- B. Sample Proposals 129
- C. Sample Tests 135

- D. Guidance for Presentations 153
 - E. Revised Paragraph from *J. Biol. Chem.* 266: 3811–3814 (1991) 155
 - F. Abstract from *Appl. Environ. Microbiol.* 63: 2647–2653 (1997) 157
 - G. Sequence Editing Tutorial 159
 - H. Peak Scanner Tutorial 173
 - I. Adding Size Standards to Peak Scanner 197
 - J. PRIMER Tutorial 203
 - K. Checklist for Grading Laboratory Reports 207
 - L. Peer Review Checklist 209
 - M. Career Advice 213
 - N. Molecular Microbiology Laboratory Preparation Manual 215
- Bibliography 253
Index 255

Full Contents

Introduction xi

- I. Writing-Intensive Course xi
 - A. Goals xi
 - B. Means xi
- II. Schedule xiii
- III. Attendance and Grading Policies xvi
 - A. Attendance xvi
 - B. Grading xvi
 - C. Partial Credit xvi
- IV. Laboratory Rules xviii
- V. Decorum xx
- VI. Flowcharts xxii
 - A. How to Prepare a Flowchart xxii
 - B. Sample Flowchart xxii
- VII. Laboratory Reports xxiv
 - A. Preparing a Laboratory Report xxiv
 - B. Sections of a Laboratory Report xxiv
- VIII. How to Evaluate Laboratory Reports xxvii
 - A. Peer Review Checklist xxvii
 - B. Criteria for Grading Laboratory Reports xxix
 - C. Checklist for Grading Laboratory Reports xxx
- IX. Proposals to Study Environmental Bacterial Populations: Writing Assignment (Class 6) xxxiii
 - A. Overview xxxiii
 - B. Site Selection xxxiii
 - C. Peer Review of Proposals to Study Environmental Bacterial Populations xxxiv
 - D. Instructions for Reviewing Proposals xxxiv
 - E. Proposal Review Forms xxxv
- X. How to Read a Journal Article xxxviii
 - A. Organization xxxviii
 - B. Order in Which to Read an Article xxxviii
 - C. Information Contained in Each Section xxxviii

XI. Writing Tips xli

- A. Begin Each Paragraph with a Topic Sentence xli
- B. Write an Informative Title xliii
- C. Avoid Wordy Phrases and the Passive Voice xliii
- D. Avoid Jargon and Colloquial Wording xlv
- E. Avoid Pretentious Wording xlv
- F. Do Not Use Nouns as Adjectives xlv
- G. Revise Sentences That Do Not Make Sense xlv
- H. Place Modifiers Properly xlv
- I. Shorten Run-on Sentences xlv
- J. Convert Fragments into Complete Sentences xlv
- K. Follow Established Conventions xlv
- L. Use *Which* and *That* Correctly xlvii
- M. Do Not End a Sentence with a Preposition xlviii
- N. Use the Correct Homophone xlviii
- O. Do Not Use the Same Word Twice in One Sentence xlviii

Acknowledgments 1

Experiment 1 Plasmid Purification and Restriction Mapping

- I. Introduction 1
- II. Background: Plasmid DNA Preparation 2
- III. Procedure 11
 - A. Purify Plasmid pKN800 DNA (Class 2) 11
 - B. Restriction of Plasmid pKN800 (Class 2) 12
 - C. Agarose Gel Electrophoresis of *Pst*I-Digested pKN800 (Class 3) 13
 - D. Transform *E. coli* Strain DH5 α with pKN800 DNA (Class 3) 14
 - E. Observe Luciferase Reporter Gene Expression (Day after Class 3) 16

- IV. Laboratory Report [Class 4 (Draft) and Class 5] 18
- V. Questions (Class 5) 20
- VI. In-Class Writing Exercise (Class 2) 22
- VII. Restriction Mapping Exercises (Class 3) 24
- VIII. In-Class Writing Exercise (Class 4) 26

Experiment 2 Affinity Purification of Histidine-Tagged FnbA Protein

- I. Introduction 29
- II. Background 30
- III. Procedure 33
 - A. Lyse Bacteria (Class 5) 33
 - B. Adsorb Histidine-Tagged FnbA Protein to Nickel-Agarose (Class 5) 33
 - C. Wash Resin and Elute Histidine-Tagged FnbA Protein (Class 5) 34
 - D. SDS-Polyacrylamide Gel Electrophoresis (Class 6) 34
 - E. Stain Gel (Class 6) 35
- IV. Laboratory Report 37
 - A. Draft (Class 8) 37
 - B. Questions (Class 9) 38
- V. Critique of Paper by Bush et al. 40

Experiment 3 Polymerase Chain Reaction and DNA Sequence Analysis of Bacterial Ribosomal RNA Genes

- I. Introduction 43
- II. Background 44
- III. Procedure 51
 - A. Sample Collection for Experiments 3 and 4 (Class 7) 51
 - B. Gram Stain and Light Microscopy (Class 8) 52
 - C. Freeze Cultures (Class 9) 53
 - D. Purify Genomic DNA (Class 9) 53
 - E. PCR Amplification of 16S rRNA Genes (Class 10) 54
 - F. Purify PCR Product (Class 11) 55

- G. Electrophoretic Analysis of PCR Product (Class 12) 56
- H. DNA Sequence Analysis (Class 14) 56
- IV. Laboratory Report [Class 16 (Draft) and Class 17] 63
- V. Questions (Class 17) 66
- VI. In-Class Writing Exercise (Class 10) 68

Experiment 4 Terminal Restriction Fragment Length Polymorphism Analysis of 16S Ribosomal RNA Genes from Uncultured Bacterial Communities

- I. Introduction 71
- II. Background 72
- III. Procedure 81
 - A. Sample Collection for Experiments 3 and 4 (Class 7) 81
 - B. Extract DNA from an Uncultured Bacterial Community (Class 7) 82
 - C. PCR Amplification of 16S rRNA Genes for Terminal Restriction Fragment Length Polymorphism Analysis (Class 14) 83
 - D. Purify PCR Product (Class 15) 84
 - E. Ligate PCR Product to Plasmid: Topoisomerase-Mediated Cloning Reaction (Class 15) 85
 - F. Transform Ligated DNA (Class 15) 85
 - G. Electrophoretic and Spectrophotometric Analyses of PCR Product (Class 15) 85
 - H. Restrict Purified PCR Product (Class 16) 86
 - I. Plasmid DNA Purification (Class 16) 86
- IV. Laboratory Report 89
- V. Questions (Class 20) 94
- VI. Writing Exercise: Editorial on Genetically Modified Crops [Class 14 (Draft) and Class 15] 96
- VII. Writing Assignment: Literature Search Problem [Class 14 (Begin) and Class 20] 98
- VIII. In-Class Writing Exercise (Class 15) 100

APPENDICES

- A. Sample Laboratory Reports 103
 - B. Sample Proposals 129
 - C. Sample Tests 135
 - D. Guidance for Presentations 153
 - E. Revised Paragraph from *J. Biol. Chem.*
266: 3811–3814 (1991) 155
 - F. Abstract from *Appl. Environ. Microbiol.*
63: 2647–2653 (1997) 157
 - G. Sequence Editing Tutorial 159
 - H. Peak Scanner Tutorial 173
 - I. Adding Size Standards to Peak
Scanner 197
 - J. PRIMER Tutorial 203
 - K. Checklist for Grading Laboratory
Reports 207
 - L. Peer Review Checklist 209
 - M. Career Advice 213
 - N. Molecular Microbiology Laboratory
Preparation Manual 215
- Bibliography 253
- Index 255