# Biotechnology: A Laboratory Skills Course, Second Edition

## **Detailed Chapter Outlines**



Below is a chapter-by-chapter outline of the student edition of Biotechnology: A Laboratory Skills Course, second edition.

The vignettes and student activities are listed at the end of each chapter outline. Please visit bio-rad.com/textbook for more information.

## **Chapter 1: Biotechnology Industry**

- 1.1 What Is Biotechnology?
  - The Biotechnology Toolkit
- 1.2 Who Uses Biotechnology?
  - Life Science Research
    - Omics and Systems Biology
  - Healthcare
    - Drug Discovery, Development, and Testing
    - Protein-Based Drug Production
    - Personalized Medicine
    - Clinical Diagnostics
  - Agriculture
  - Food
  - Industrial Manufacturing
  - Biofuels
  - Mining
  - Pollution Monitoring and Waste Management
  - Conservation
  - Biodefense
  - Forensics
  - Human Origins
  - Nanotechnology
- 1.3 The Biotechnology Industry
  - What Is a Biotechnology Company?
  - Biotechnology Product Development
  - Funding Biotechnological Advancement
  - Patents
- 1.4 Governmental Regulation of Biotechnology
  - Regulation of Genetically Modified Organisms
  - Regulation of Products in Healthcare
  - Preclinical Research
  - Clinical Trials
  - Regulation of Animal Research
  - Regulation of Human Research
  - Regulation of Waste Disposal
  - Regulation of Nanotechnology
  - Workplace Safety Regulation

- 1.5 Industry Practices
  - Good Laboratory Practice
  - Good Manufacturing Practice
- 1.6 Biotechnology Company Structure
- 1.7 Careers in Biotechnology
  - Careers in the Laboratory
    - Laboratory Technician
    - Quality Assurance Technician
    - Research Associate
    - Research and Development Scientist
    - Clinical Scientist
    - Engineer
  - Non-Laboratory Career

#### **Chapter 1 Vignettes**

Bioethics - Biotechnology: Good or Bad?

Biotech in the Real World — Affecting Government Policy

Biotech in the Real World — Bio-Rad: Then and Now

Careers in Biotech — Timothy Balmorez, Chemist, Bio-Rad Laboratories, Inc.

How To... Write an SOP

## **Chapter 2: Laboratory Skills**

- 2.1 Laboratory Safety
  - Operating Safely in a Laboratory
  - Safety Training
  - Personal Protective Equipment
  - Chemical Safety
  - Biological Safety
  - General Laboratory Safety
  - Waste Disposal
  - Cleanrooms
- 2.2 Laboratory Notebooks
  - Laboratory Notebook Structure
  - Components of a Laboratory Notebook Entry



#### 2.3 Laboratory Equipment

- Measuring volumes
  - Graduated Cylinders
  - Volumetric Flasks
  - Vacuum-Assisted Pipetts
  - Micropipets
  - Burettes
- Liquid Containers
  - Erlenmeyer Flasks
  - Reagent Bottles
- Measuring Mass
  - Balances
- Measuring Temperature
  - Thermometers
- Measuring pH
  - pH Meters
- Calibration
- Washing Glassware
- Disinfecting and Sterilizing
  - Autoclaving
  - Disinfecting Surfaces with Bleach
  - Disinfecting Surfaces with Alcohol
  - Disinfecting Using a Microwave Oven
  - Disinfecting with Germicidal UV Light
  - Sterilizing Solutions
- Labeling

### 2.4 Numerical Data

- Significant Figures
- Scientific Notation
- Units of Measure
  - Metric Prefixes

#### 2.5 Preparing Solutions

- Percent Solutions
  - Mass per Volume Percent Solutions
  - Volume per Volume Percent Solutions
  - Percentages, Ratios, and Proportions
  - Diluting Percent Solutions
- Using Stock Solutions Given in Terms of "x"
- Molar Solutions
  - The Mole
  - Molarity
  - Making Molar Solutions
    - a. Proportional Method
    - b. Unit Cancellation Method
- Making Compound Molar Solutions
- Diluting Molar Solutions
- Preparing Small Volume Dilutions
- Normality

#### **Chapter 2 Vignettes**

Bioethics - Waste Disposal

Biotech in the Real World — Who Invented Claritin? How Laboratory Notebooks Played Their Part

Careers in Biotech — Joshua Moore, Bio-Containment Protocol Support Supervisor, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

How To... Use an Adjustable-Volume Micropipet

#### **Chapter 2 Activities**

- 2.A DNA Extraction and Precipitation
- 2.B Pipetting
- 2.C Kool-Aid Column Chromatography
- 2.D Making Solutions
- 2.E Titration
- 2.F Writing a Standard Operating Procedure

## Chapter 3: Microbiology and Cell Culture

- 3.1 Microbiology and Cell Biology
  - Three Domains of Life
  - Microorganisms and History
- 3.2 Bacteria
  - Names and Shapes of Bacteria
  - Bacterial Environments
- 3.3 Uses of Bacteria in Biotechnology
  - Food Production
  - Protein Production
- 3.4 Culturing Bacteria in the Laboratory
  - Growth Media
    - Solid Media
    - Antibiotic Selection
    - Liquid Media
- 3.5 Microbiological Techniques
  - Tools
  - Aseptic Technique
  - Using Streak Plate Technique to Isolate Single Colonies
  - Labeling Media
  - Quantifying Bacteria
    - Serial Dilution and Plate Counts
    - Optical Density of Bacteria
  - Identification of Bacteria
- 3.6 Eukaryotic Cells
  - Organelles
  - Other Eukaryotic Cells

- 3.7 Uses of Eukaryotic Cells in Biotechnology
  - Fermentation
  - Protein Production
  - Stem Cells
- 3.8 Eukaryotic Cell Culture
  - Growth Media
  - Environment
  - Sterility
  - Visualization

#### **Chapter 3 Vignettes**

Bioethics — Should Human Embryos Be Used for Research?

Biotech in the Real World — Artificial Life!

Careers in Biotech — Katie Dalpozzo, Laboratory Research Associate, Bavarian Nordic

How To... Use Aseptic Technique to Transfer Bacteria

#### **Chapter 3 Activities**

- 3.A Making Microbiology Media
- 3.B Disk Diffusion Test (Modified Kirby-Bauer Test)
- 3.C Microbes and Health: An Illustration of Koch's Postulates
- 3.D Gram Staining
- 3.E Quantifying Bacterial Numbers
- 3.F Staining Eukaryotic Cells

## **Chapter 4: DNA Structure and Analysis**

- 4.1 Molecular Biology
  - The Central Dogma of Molecular Biology
- 4.2 DNA Structure
- 4.3 Recombinant DNA Technology
  - Restriction Enzymes
  - Ligases
  - Nontraditional Cloning Techniques
    - Golden Gate Assembly
    - Gibson Assembly
    - TOPO (TA) Cloning
  - Using Enzymes in the Laboratory
  - CRISPR-Cas9 Technology
    - How CRISPR works
    - Applications of CRISPR Technology
      - a. Agriculture
      - b. Industry
      - c. Public Health
      - d. Medicine

- 4.4 DNA Analysis Techniques
  - Horizontal Agarose Gel Electrophoresis
  - Agarose Gels and Running Buffer
  - Standards for DNA Electrophoresis
  - Equipment
    - Horizontal Gel Electrophoresis Chambers
    - Power Supplies
  - Running an Agarose Gel
  - Agarose Gel Staining and Imaging
- 4.5 Restriction Enzymes as Forensic Tools
  - Restriction Fragment Length Polymorphisms
  - Southern Blotting

#### **Chapter 4 Vignettes**

Bioethics — Personal Genetic Information

Biotech in the Real World - Fighting Crime with DNA

Careers in Biotech — Elisa Ciullo, Sr. Supervisor, Clinical Filing Operations, Genentech

How To... Set Up a Restriction Digest

#### **Chapter 4 Activities**

- 4.A Restriction Site Prediction Using NEBcutter
- 4.B Casting Agarose Gels
- 4.C Dye Electrophoresis
- 4.D Restriction Digestion and Analysis of Lambda DNA
- 4.E Forensic DNA Fingerprinting
- 4.F Plasmid Mapping

# Chapter 5: Bacterial Transformation and Plasmid Purification

- 5.1 History of Bacterial Transformation and Plasmids Bacteria
  - Plasmid Structure
  - Features of Plasmids
  - Using Plasmids in Biotechnology
  - Transcriptional Regulation of Plasmids
  - Genetically Engineering the pGLO Plasmid
  - Plasmids for Eukaryotic Expression
- 5.2 Transforming Cells
  - Selection of Transformed Cells
  - Transformation Efficiency
- 5.3 Plasmid Purification and Quantitation
  - Growing Bacteria in a Liquid Culture
  - Purifying Plasmid DNA from a Culture
  - DNA Quantitation
    - Gel Quantitation
    - Spectrophotometric Quantitation
    - Quantitation of DNA with a Fluorometer

#### **Chapter 5 Vignettes**

Bioethics — A World without Antibiotics?

Biotech in the Real World - Biotech on the Pharm

Careers in Biotech — Denise Gangadharan, PhD, Associate Director for Science, Centers for Disease Control and Prevention

How To... Transform E. coli Using Electroporation

How To... Use a Microcentrifuge

#### **Chapter 5 Activities**

- 5.A Bacterial Transformation with S3 Plasmid
- 5.B Bacterial Transformation with pGLO Plasmid
- 5.C Purification of S3 and pGLO Plasmids
- 5.D DNA Quantitation

## **Chapter 6: The Polymerase Chain Reaction**

- 6.1 Invention of PCR
- 6.2 What Is PCR?
  - Three Stages of PCR
    - Denaturation
    - Annealing
    - Extension
  - PCR and DNA Replication
  - Components of a PCR Reaction
  - Setting Up a PCR Reaction
    - Master Mixes
  - Analysis of PCR Products
- 6.3 Thermal Cyclers
- 6.4 Types of PCR
  - Real-Time, or Quantitative, PCR
  - Digital PCR
  - Reverse Transcription PCR
  - Multiplex PCR
  - Degenerate PCR
  - Nested PCR
  - Fast PCR
  - Isothermal PCR
  - Random Amplification of Polymorphic DNA
- 6.5 PCR Optimization
  - Quality of Template DNA
  - Primer Design
  - Cycling Parameters
  - Magnesium Concentration

- 6.6 Techniques Based on PCR
  - DNA Microarrays
  - DNA Sequencing
    - Primer Sequences
  - Next-Generation Sequencing
    - Sequencing by Synthesis
    - Pyrosequencing
    - Ion Semiconductor Sequencing
    - Sequencing by Ligation
  - Sequence Data Analysis Using Bioinformatics
- 6.7 Real-World Applications of PCR
  - PCR in Medicine
  - PCR in Agriculture
  - PCR in Forensics
  - PCR in Paternity Testing
  - PCR in Human Migration
  - PCR in Wildlife Conservation

#### **Chapter 6 Vignettes**

Bioethics — Forensic DNA Databases — Is Your Privacy Protected?

Biotech in the Real World — A Universe of Viruses

Careers in Biotech — Dora Barbosa, Research Associate, University of California San Francisco

How To... Program a Thermal Cycler

#### **Chapter 6 Activities**

6.A STR PCR Analysis

- 6.B GMO Detection by PCR
- 6.C Detection of the Human PV92 Alu Insertion
- 6.D Fish DNA Barcoding

## **Chapter 7: Protein Structure and Analysis**

- 7.1 Protein Synthesis
  - Bacterial Transcription
  - Eukaryotic Transcription
  - Translation
  - Posttranslational Modifications
- 7.2 Protein Structure
  - Amino Acids
  - Protein Folding
- 7.3 Proteins in Biology
  - Fibrous Proteins
  - Globular Proteins
    - Enzymes
- 7.4 Proteins in Biotechnology
  - Proteins in Industry
  - Proteins in Healthcare
  - Proteins as Tools in Life Science Research

#### 7.5 Methods of Protein Analysis

- Protein Quantitation
  - Biuret Test
  - Lowry Assay
  - Bradford Assay
- Protein Properties Used in Analysis
  - Protein Size
  - Protein Charge
- Protein Mass Spectrometry
- Protein Electrophoresis
  - Polyacrylamide Gels
  - Electrophoresis Running Buffer
  - Discontinuous Buffer Systems
  - Visualizing Proteins in Polyacrylamide Gels
- Other Types of PAGE
  - Native PAGE
  - Isoelectric Focusing
  - Two-Dimensional PAGE
  - Nucleic Acid Separation
- Protein Chromatography
  - Size Exclusion Chromatography
  - Hydrophobic Interaction Chromatography
  - Ion Exchange Chromatography
  - Affinity Chromatography
  - Analyzing the Results of Chromatography
  - Scaling Chromatography
- Protein Analysis Using Bioinformatics

#### 7.6 Protein Production in Industry

- Protein Production
- Quality Control in Protein Production

### **Chapter 7 Vignettes**

Bioethics - Editing Genes to Cure Disease

Biotech in the Real World - Vaccines in Bananas

Careers in Biotech — Scott Chilton, Marketing Manager, Maravai Life Sciences

How To... Set Up a Vertical Electrophoresis System

#### **Chapter 7 Activities**

- 7.A Protein Quantitation Using the Bradford Assay
- 7.B Size Exclusion Chromatography
- 7.C GFP Purification by Hydrophobic Interaction Chromatography
- 7.D SDS-PAGE of Fish Muscle
- 7.E Biofuel Enzyme Assay
- 7.F Exploring Bioinformatics with GFP

## **Chapter 8: Immunological Applications**

- 8.1 The Immune System
  - Cells of the Immune System
  - Antibodies
  - Immune Response

#### 8.2 Antibodies as Tools

- Polyclonal Antibodies
- Monoclonal Antibodies
- Humanized Monoclonal Antibodies for Medical Use

#### 8.3 Immunoassays

- Labeling and Detecting Antibodies
- Enzyme-Linked Immunosorbent Assay (ELISA)
  - ELISA for Direct Antigen Detection
  - ELISA for Indirect Antigen Detection
  - Sandwich ELISA for Antigen Detection
  - ELISA for Antibody Detection
  - Instruments for ELISA
- Ouchterlony Double Diffusion Assay
- Western Blotting
  - Electroblotting
  - Immunodetection
  - Western Blotting in Diagnostic Testing
- Using Antibodies to Study Cells and Tissues
- High-Throughput Immunoassays
  - Multiplex Bead Assays
  - Fluorescence-Activated Cell Sorting

#### **Chapter 8 Vignettes**

Bioethics - Testing for HIV Status at Home

Biotech in the Real World — Using Immunodetection to Save the Panda

Biotech in the Real World — Car T Cell Therapy — A New Horizon in Cancer Treatment

Careers in Biotech — Sophy Wong, MD, Associate Clinical Professor of Medicine, University of California San Francisco, CA; Medical Director for HIV ACCESS and AETC

How To... Select an Antibody

#### **Chapter 8 Activities**

- 8.A Ouchterlony Double Immunodiffusion Assay
- 8.B Serum Antibody Detection by ELISA
- 8.C Quantitative ELISA
- 8.D Western Blotting

## **Chapter 9: Research Projects**

- 9.1 What Is Research?
  - Deductive and Inductive Research
  - Conducting Research in the Real World
  - Peer Review
  - Sharing of Scientific Information
    - Peer-Reviewed Publications
    - Conferences, Meetings, Seminars, and Posters
- 9.2 Student Research Projects
  - Whole Class Projects
  - Group Projects
  - Individual Projects
  - Collaborating with Scientists
  - Venues for Conducting Research or Entering Competitions
    - High School Research
    - Undergraduate Research
  - Choosing a Research Project
  - Scoping a Research Project
  - Planning a Project
  - Performing Background Research
    - Internet
    - Libraries
    - Peer-Reviewed Journal Articles
    - Mentors
  - Experimental Design
    - Methods
    - Controls
    - Trials and Sample Size
  - Data Analysis
    - Example of Data Analysis
    - Calculating the Mean
    - Calculating the Standard Deviation
    - Drawing Conclusions from Statistical Data
  - Funding Research Projects
- 9.3 Communicating Research
  - Posters
  - Research Papers
  - Oral Presentations

#### 9.4 Project Ideas

- Research Tools and Techniques
- Agricultural and Animal Research
- Food Science
- Humans and Health
- Forensics
- Energy and the Environment

#### 9.5 Considerations and Tips for All Laboratory Projects

- Microbiology
- Animal Research
- Research on Human Subjects
- Molecular Biology
  - Guidelines for Working with Recombinant DNA
  - Restriction Enzymes
  - PCR
  - Extracting DNA
  - Primer Design
  - Master Mixes
  - Determining the Annealing Temperature
  - Ligation and Transformation
  - DNA Sequencing
- Proteins
  - Obtaining Antibodies
  - Optimizing Immunoassays
  - Enzymes and Substrates
  - Chromatography

#### **Chapter 9 Vignettes**

Bioethics - Clinical Trials and Data Fraud

Biotech in the Real World — Regeneron Science Talent Search

Careers in Biotech — Sunny Choe, PhD, Senior Medical Scientist, Gilead Sciences, Inc.

How To... Read a Journal Article

#### **Appendices**

- A: Fast Gel Protocol
- B: Alternative Staining Methods for Agarose Gels
- C: Stain-Free SDS-PAGE Gel Imaging
- D: Glossary
- E: Laboratory Skills Assessment Rubric
- F: Laboratory Notebook and Project Rubrics

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