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 Pipets
  - 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

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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

- 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

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- 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

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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

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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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Bulletin 7123 Ver B 25-759503 0326 Sig 0226