Biotechnology: A Laboratory Skills Course will guide you and your students through the exciting world of biotechnology!
The polymerase chain reaction (PCR) has revolutionized the study of living things. Invented by Kary Mullis in 1983, PCR has become a cornerstone of molecular biology research. It is the basis of the Human Genome Project, revolutionizing molecular biology and genetic engineering. Using PCR, a small sequence of DNA of a few hundred base pairs can be found within a genome of billions of base pairs. Billions of copies of the sequence are then generated, making the DNA sequence available for study and manipulation. This technique has transformed our understanding of life and has opened up new avenues of research.

Summary

The polymerase chain reaction (PCR) has revolutionized the study of living things. Invented by Kary Mullis in 1983, PCR has become a cornerstone of molecular biology research. It is the basis of the Human Genome Project, revolutionizing molecular biology and genetic engineering. Using PCR, a small sequence of DNA of a few hundred base pairs can be found within a genome of billions of base pairs. Billions of copies of the sequence are then generated, making the DNA sequence available for study and manipulation. This technique has transformed our understanding of life and has opened up new avenues of research.

Chapter overview

Four types of vignettes show how biotechnology concepts covered in the chapter play a role in our daily lives. Vignette topics include discussions about bioethics, careers, spotlights on key skills, and real-life case studies.
Activities implement the techniques described in the background information. Early activities focus on building basic skills, while later activities use those basic skills as a foundation for more advanced techniques.

Laboratory skills are acquired by performing the activity. The requirements necessary to claim proficiency in those skills are described in the Laboratory Skills Assessment Rubric in Appendix E.

Graphics illustrate the hands-on activities to help students learn techniques.

Step-by-step protocols lead students through procedures and provide guidance on results analysis.

Activity 3.4 Gram Staining

Overview
In 1964, a technique to differentiate between the two types of bacteria was invented by the Danish scientist Hans Christian Gram. This technique utilizes a step-by-step staining procedure using different dyes and is still one of the best tests used when trying to identify unknown bacteria. Gram-positive bacteria have a very thin layer of peptidoglycan composed of layers of carbohydrates, while Gram-negative bacteria have a thicker peptidoglycan layer composed of layers of phospholipid membranes. And the cytoplasmic wall does not line up and absorb crystal violet dye, which is used as a counterstain, makes Gram-negative bacteria appear purple (see Figure 3.3).

This activity will allow you to stain Gram-positive and Gram-negative bacteria. You will observe the stained bacteria using a microscope and determine the shape and size of the bacteria under various conditions: whether they are gram-positive or negative.

Tips and Tricks

- Avoid drying the specimen. The water will evaporate, and the specimen will be damaged.
- Plan ahead for staining. The specimens should be prepared before the lesson begins.

Safety Precautions

- Wear proper protective clothing and equipment, including goggles and gloves, when handling any of the chemicals used in this activity.
- Do not handle or use any of the chemicals without proper supervision.

Research Questions

1. What’s the basic staining process of Gram-negative and Gram-positive bacteria?
2. How does the staining process work in determining the type of bacteria?
3. What is the difference between Gram-positive and Gram-negative bacteria?

Prelab focus questions ensure students' understanding of the activity, and postlab focus questions help students analyze their results and generate conclusions.

Assessment rubrics help students understand what is expected of them and how to proficiently complete a task.

Appendix E: Laboratory Skills Assessment Rubric

<table>
<thead>
<tr>
<th>Activity</th>
<th>Skill</th>
<th>Practice</th>
<th>Developing</th>
<th>Proficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>Follow laboratory procedures</td>
<td>Student may not understand the importance of following proper procedure. Procedure is performed out of order or in making multiple errors. Errors can be corrected.</td>
<td>Student understands the importance of following proper procedure. Procedure is performed in the appropriate sequence and all errors are corrected.</td>
<td>Student demonstrates the importance of following proper procedure. Procedure is performed in the appropriate sequence and no errors are made.</td>
</tr>
<tr>
<td>2.1</td>
<td>Select and use proper PPE</td>
<td>Student may not understand the importance of using PPE. Student is not able to follow the correct PPE. Procedure is performed with incorrect PPE or does not perform within the time limit.</td>
<td>Student understands the importance of using PPE. Procedure is performed in the appropriate time frame.</td>
<td>Student demonstrates the importance of using PPE. Procedure is performed in the appropriate time frame and no errors are made.</td>
</tr>
<tr>
<td>2.3</td>
<td>Extract DNA from cells</td>
<td>Student may not understand the importance of DNA extraction. Student performs the procedure incorrectly, uses incorrect steps, or performs the procedure out of order, resulting in no viable DNA.</td>
<td>Student understands the sequence of DNA extraction. Student performs the procedure correctly and has viable DNA.</td>
<td>Student demonstrates the ability to extract DNA accurately and in a timely manner.</td>
</tr>
<tr>
<td>2.3</td>
<td>Preprocess DNA</td>
<td>Student may not understand the importance of DNA preprocessing. Student may perform the protocol incorrectly and DNA is not viable.</td>
<td>Student understands the importance of DNA preprocessing. Student handles the sample correctly leading to viable DNA.</td>
<td>Student demonstrates the ability to use a centrifugal pump correctly. DNA is easily viable and may be present in solution or in a liquid. Students may be able to use a centrifugal pump.</td>
</tr>
<tr>
<td>2.3</td>
<td>Process DNA</td>
<td>Student may not understand the importance of DNA processing. Student may perform the protocol incorrectly and DNA is not viable.</td>
<td>Student understands the importance of DNA processing. Student handles the sample correctly leading to viable DNA.</td>
<td>Student demonstrates the ability to use a centrifugal pump correctly. DNA is easily viable and may be present in solution or in a liquid. Students may be able to use a centrifugal pump.</td>
</tr>
</tbody>
</table>

Student Textbook Activities and Assessment Rubrics
Microbiology and Cell Biology

Cell biology is the study of cells. Microorganisms are comprised of single cells or small clusters of cells; in contrast, human tissues are made of millions of cells working in concert. Cells are either prokaryotic or eukaryotic. Eukaryotic cells have a nucleus and other membrane-bound organelles. Prokaryotic cells do not have a nucleus or membrane-bound organelles and are much smaller than eukaryotic cells (see Figure 3.1).

Part of cell biology, microbiology is the study of microorganisms and their effects on other living organisms. The terms microbe and microorganism refer to organisms that must be viewed at the microscopic level such as bacteria, yeast, algae protozoa, fungi, and viruses. (Note: There is no consensus on whether viruses are living organisms; however, since viruses can be viewed with a microscope, they are often included under the umbrella of microbiology.) The majority of naturally occurring microbes are harmless, and many are used in biotechnology to benefit mankind. However, harmful microbes cause many types of disease affecting people, animals and plants.

Understanding cell biology and microbiology is necessary in biotechnology because cells do much of the work of biotechnology, such as making recombinant DNA and proteins and because cells are the targets of many biotechnological products. For example, drugs are engineered to target specific proteins on the plasma membranes of human cells.

Three Domains of Life

Life on earth is organized into three domains: Archaea, Bacteria, and Eukarya (see Figure 3.2). Archaea and Bacteria are prokaryotes. Eukarya include yeast, algae, and humans. There are many more prokaryote species than eukaryote species on earth, and there are more Bacteria than Archaea.

Archaea were not recognized as a separate domain until the 1970s. Currently, there are no known human diseases caused by Archaeans. Archaea are found in most habitats on earth including the human digestive system. Many Archaea live in very harsh environments such as deep ocean volcanic vents or salt lakes and are called extremophiles. Archaea are being investigated as sources of enzymes that could be used in harsh manufacturing or experimental conditions (for example, enzymes used to process food at high temperatures). Bacteria and eukaryotic cells are described in detail in this chapter.

Microorganisms and History

Throughout history, man has been afflicted by diseases. The impact of diseases such as malaria, anthrax, and plague has changed the course of history. Analyses of the remains of King Tutankhamun in Egypt (see Figure 3.3) indicate that malaria, which is caused by a single-celled eukaryotic protozoan from the genus Plasmodium, contributed to the king’s death more than 3,000 years ago. Malaria also had a role in the decline of the Roman Empire 1,500 years ago. Anthrax is caused by the Bacillus anthracis bacterium whose spores are common in soil. Anthrax is thought to be the source of the fifth plague of Egypt mentioned in the Book of Exodus in the Bible that devastated Egyptian livestock. Plague, which is caused by the bacterium Yersinia pestis, is believed to be the source of the Black Death that killed millions of people during the Middle Ages, and resulted in the loss of 30–60% of Europe’s population.

Today, malaria kills around 1 million people each year, most of whom are children in sub-Saharan Africa. A major effort is under way by the World Health Organization (WHO) to reduce the number of people killed by malaria. Plague infects thousands of people worldwide each year, but advances in antibiotics and other treatments, have dramatically reduced fatalities. Anthrax rarely infects humans (see Figure 3.4) but remains a threat to society because it can be used as
**How To...**

**Use an Adjustable-Volume Micropipet**

The volume of a micropipet is changed by twisting either a ridged cylinder on the micropipet handle or the top of the plunger, depending on the style of micropipet. When the volume is changed, the new volume is displayed on the readout dial. Figure 2.12 demonstrates how to read the volume on three common micropipet sizes. A micropipet is always used with a pipet tip. The end of the micropipet is inserted into the open end of the tip and tapped gently while the tip is in the box. This method ensures that the end of the tip is not touched. Tips are removed by pressing the tip ejector button.

**Burettes**

A burette is a long, glass, graduated tube that is similar to a graduated pipet; however, it is filled by pouring a solution into the top and emptied via a stopcock at the base (see Figure 2.11). A burette is usually held upright with a stand and clamp. This glass tube is commonly used during titration when the volume of liquid to be dispensed is variable and needs to be measured after dispensing. A measurement is taken before and after dispensing the liquid, and the volume dispensed is then calculated.

\[ V_{\text{final}} - V_{\text{initial}} = V_{\text{delivered}} \]

where \( V \) = volume

Burettes are usually used to accurately measure volumes between 10–100 ml; for example, a 25 ml burette can have an accuracy of ± 0.06 ml.

**Liquid Containers**

**Erlenmeyer Flasks**

An Erlenmeyer flask has slanted sides and a narrow opening that facilitate swirling and mixing of the contents without spilling. (see Figure 2.14). Erlenmeyer flasks are typically not used for measuring, although the graduations on the flasks provide a rough guide for measuring liquids within approximately 5% of the target volume. Erlenmeyer flasks with the capacity to handle from 25 ml to 4 L are commonly found in a laboratory. Erlenmeyer flasks with fire-polished tops are primarily used for mixing and moving solutions from one location to another; these flasks can be used for short-term storage when covered with Parafilm or another impermeable cover. Erlenmeyer flasks with a culture top or screw cap top are used to grow liquid cultures of microorganisms. Such flasks may have baffles added to the bottom to aid in the agitation and oxygenation of the liquid medium as it is swirled by the shaking incubator. Erlenmeyer flasks may also have ground glass stoppers that enable the contents to be stored temporarily.
Activity 4.5 Forensic DNA Fingerprinting Protocol

Part 1: Setting Up the Digestion Reactions

1. Label colored microcentrifuge tubes:
   - green tube  CS (crime scene)
   - blue tube  S1 (suspect 1)
   - orange tube  S2 (suspect 2)
   - violet tube  S3 (suspect 3)
   - pink tube  S4 (suspect 4)
   - yellow tube  S5 (suspect 5)

   Label all the tubes with your initials and date, and place them in the microcentrifuge tube rack.

2. Using a fresh tip for each sample, pipet 10 µl of each DNA sample into the matching colored microcentrifuge tube. Make sure each sample is transferred to the bottom of the tube.

3. Using a fresh tip each time, pipet 10 µl of enzyme mix (ENZ) into the bottom of each tube. Pipet up and down carefully to mix.

4. Tightly cap the tubes, and mix the components by gently flicking the tubes with your finger. Collect the samples at the bottom of the tubes by tapping the tubes gently on the table or by pulse-spinning them in a microcentrifuge.

5. Incubate the digestion reactions for 45 min at 37°C or overnight at room temperature.

6. After the incubation, store the samples at 4°C until the next laboratory period. Samples can be stored for 1 month at 4°C. If there is sufficient time, proceed to running the gel.

7. 1x TAE electrophoresis buffer and a 1% TAE agarose gel is required for the next part of the activity. If necessary, prepare 1x TAE (refer to part 1 of Activity 2.4) and a 1% TAE agarose gel (refer to Activity 4.2).

Part 2: Running the Gel

1. If condensation has collected on the lids of the tubes, collect the samples at the bottom of the tubes by tapping the tubes gently on the bench or by pulse-spinning them in a microcentrifuge.

2. Using a fresh tip for each sample, pipet 5 µl of the 5x sample loading buffer (SLB) into each tube. Cap the tubes and mix by gently flicking the tubes with your finger. Collect the samples at the bottom of the tubes by tapping them gently on the bench or by pulse-spinning them in a centrifuge.
Teacher Supplement: Step-by-step activity preparation

Teacher Supplement Monoclonal and Cell Culture

Activity 3.5 Quantifying Bacterial Numbers

Quantifying Bacterial Numbers
In this activity, students will make a serial dilution with a dilution factor of 10 to 100. A 100 μL volume of bacterial culture will be used to prepare a 10-fold dilution of the culture. The 10-fold dilution will be used to prepare a 100-fold dilution, and so on. The number of bacteria in the original culture will be determined using a hemocytometer and a Bichow counting chamber.

Activity Timelines

Activity Date

ACTIVITY 3.5

Activity Timelines

| Activity Date | Process
|---------------|---------|
| Week 3 | Prepare the bacterial culture and count the number of bacteria using a hemocytometer and a Bichow counting chamber.

Anticipated Results

Students should see a decrease in the number of colonies formed across the dilution series. If the last plate has less than 30 colonies, the previous plates should have approximately 100 colonies each. Students should be able to determine the number of bacteria in the original culture by analyzing the dilution series.

Analysis of Results

Students should be able to determine the number of bacteria in the original culture by analyzing the dilution series. If the last plate has less than 30 colonies, the previous plates should have approximately 100 colonies each. Students should be able to determine the number of bacteria in the original culture by analyzing the dilution series.

Bacterial Growth

Bacteria grow in colonies on solid media, and their growth can be monitored by counting the number of colonies on each plate.

Activity 3.6 Quantifying Bacterial Numbers

Bacteria per ml of the original culture = # of CFUs x dilution factor

Bacteria per ml of the original culture = 4 x 10^6 CFUs x 10

Bacteria per ml of the original culture = 4 x 10^7 CFUs

Students may obtain different results since they may have different plates for each student. This variation is acceptable because the results are plotted graphically and the trends are analyzed.

This supplement provides implementation guidance:
- Tips
- Safety
- Activity timelines
- Stopping points
- Advanced preparation procedures
- Answers to prelab and postlab questions

Instructional Videos and Presentations:

Digital resources support implementation

30 technique videos provide classroom guidance:
- Demonstrations of key techniques
- Highlights of important safety points

43 presentations in PowerPoint format provide classroom instruction for:
- Chapter backgrounds
- Textbook laboratory activities

The teacher supplement is a full-size bound book with more than 200 pages to help you prepare and teach the activities.
Chapter Titles
Chapter 1 – Introduction to Biotechnology
Chapter 2 – Laboratory Skills
Chapter 3 – Microbiology and Cell Culture
Chapter 4 – DNA Structure and Analysis
Chapter 5 – Bacterial Transformation and Plasmid Purification
Chapter 6 – The Polymerase Chain Reaction
Chapter 7 – Protein Structure and Analysis
Chapter 8 – Immunological Applications
Chapter 9 – Research Projects

Laboratory Notebook
Students can document their laboratory activities and begin building their skills resume. 192 pages, contains fields considered to be industry standard for proper lab notebook documentation.

Laboratory Technique Videos
30 instructional videos that highlight how to master essential laboratory techniques.

Chapter Background and Activity Presentations in PowerPoint Format
43 presentations can be used to present chapter backgrounds and workflow for chapter activities. The technique videos and presentations are available as a DVD with the teacher edition.

J. Kirk Brown taught at Tracy High School in Tracy, CA, for more than 20 years. Integrating his teaching with inspiring hands-on laboratory experiences in his International Baccalaureate Biology and Biotechnology courses, he’s helped hundreds of students develop into savvy biologists. Kirk also helped to found the Agricultural/Scientific Academy at Tracy High School, which allows students to apply science learned in the classroom to the community they live in. Currently Kirk serves as the Director of Science and Special Projects for the San Joaquin County Office of Education in Stockton, CA.

Kirk’s passion for education extends into the postsecondary level through his work as an adjunct associate professor at San Joaquin Delta College, where he teaches courses in core biology and fundamentals of biotechnology. His collaborations with the California Department of Education, Lawrence Livermore National Laboratory (LLNL), San Joaquin County Office of Education, Access Excellence (Genentech), and the Exploratorium, and his ongoing partnership with Bio-Rad Laboratories, have led to significant teacher and student advancements.

Catalog # Description
166-1051EDU Laboratory Notebook, ISBN 978-0-9832396-2-8
166-1052EDU Supplementary Materials DVD Set

Parafilm is a registered trademark of American National Can Co. PowerPoint is a registered trademark of Microsoft Corporation.