Put the power and excitement of biotechnology at your students’ fingertips.

Biotechnology: A Laboratory Skills Course
Second Edition
New edition

Biotechnology: A Laboratory Skills Course, second edition, by J. Kirk Brown
integrates concepts and hands-on laboratory activities together with real-world applications for your biotechnology course.

Content Overview

Chapter 1 The Biotechnology Industry
Explore the biotechnology industry, its history, and how it is regulated by government agencies, including the FDA, USDA, and EPA. Students will learn about standard practices used in industry to consistently manufacture quality biotechnology-related products.

Chapter 2 Laboratory Skills
Learn the fundamentals of working in a laboratory, such as maintaining a laboratory notebook, laboratory safety, using equipment, performing calculations, and waste disposal. Laboratory skills include DNA extraction, pipetting, calculating dilutions, making solutions, titration, and writing SOPs.

Chapter 3 Microbiology and Cell Culture
Aseptic technique allows researchers to work with prokaryotic and eukaryotic cell cultures for a wide array of research applications. Laboratory skills include media preparation, culturing bacteria, Gram staining, streaking plates, serial dilutions, and eukaryotic cell staining.

Chapter 4 DNA Structure and Analysis
Understanding the tools used to manipulate DNA is key to molecular biology. Students will learn the basics of DNA structure along with manipulation techniques and tools, including restriction enzymes, ligases, advanced cloning techniques, and CRISPR technology. Laboratory skills include restriction enzyme digestion, horizontal agarose gel electrophoresis, DNA fingerprinting, and plasmid mapping.

Chapter 5 Bacterial Transformation and Plasmid Purification
Discover why molecular biologists use plasmids, how antibiotics work, and how genes are regulated. Students will take their first steps to becoming genetic engineers with laboratory skills including transformation, plasmid purification, DNA quantitation, and spectrophotometry.

Chapter 6 The Polymerase Chain Reaction
PCR is a cornerstone technology that revolutionized the field of molecular biology. It has continued widespread applications in agriculture, forensics, wildlife conservation, DNA sequencing, and more. Students will perform PCR to identify suspect DNA, detect genetically modified material in foods, determine human relatedness, and barcode fish species.

Chapter 7 Protein Structure and Analysis
The structure of a protein provides vital clues about its function. Students will learn about protein translation, protein production, and the role of proteins in drug discovery. Laboratory skills include chromatography, SDS-PAGE, protein quantitation, enzyme assays, and protein sequence bioinformatics.

Chapter 8 Immunological Applications
Immunoassays are powerful techniques used in research and clinical labs to determine the presence of a target. Clinically, ELISAs and western blots help determine diagnoses, such as pregnancy or HIV. Laboratory skills include using antibodies, ELISAs, and western blots.

Chapter 9 Research Projects
The skills learned in chapters 1–8 culminate in independent research projects for the whole class or for individual students. Students will integrate their experiences to formulate a hypothesis, design experiments, troubleshoot, conduct research, analyze data and develop conclusions. More than 100 research project ideas are included.
About the Author

J. Kirk Brown is the Director of STEM Programs at the San Joaquin County Office of Education, in Stockton, CA. He is a National Board–certified teacher and the former Science Department Chair at Tracy High School, in Tracy, CA, where he taught for 25 years. As an adjunct associate professor at San Joaquin Delta College, in Stockton, CA, he taught courses in Core Biology and Fundamentals of Biotechnology, and was the lead instructor at the Edward Teller Education Center at the Lawrence Livermore National Laboratory (LLNL), in Livermore, CA. Currently he leads a team of professionals that conducts teacher professional learning programs and develops STEM-related opportunities for students in central California.

Kirk has inspired generations of students and has seen his students become leaders in their fields. Many of Kirk’s former students have attended high-profile universities, received science, technology, engineering, and math (STEM) degrees at all levels, become science teachers, and pursued a wide range of careers. Many have been selected for prestigious honors themselves. As a lifelong mentor, Kirk maintains connections with his former students, building bridges among current and past students.

Now Aligned with BACE

The Biotechnician Assistant Credentialing Exam (BACE) allows students to demonstrate mastery of knowledge and skill sets that are valued by the biotechnology industry and enable growth opportunities for students in higher education.

As the demand for workforce credentialing opportunities grows, a comprehensive textbook that combines theory with relevant, hands-on activities reflective of both the academic and industrial workplaces is essential.
Chapter 6: The Polymerase Chain Reaction

6.1 Invention of PCR

A revolutionary technique, PCR has revolutionized the field of molecular biology. It allows for the amplification of specific DNA sequences, enabling the detection and quantification of a wide range of biological samples. PCR was first described by Kary Mullis in 1983 and has since become an indispensable tool in the laboratory.

6.2 What is PCR?

PCR is a process that uses enzymes to increase the number of copies of a specific DNA segment. The technique involves denaturing the double-stranded DNA, annealing primers to the DNA strands, and then extending the primers using DNA polymerase enzymes. The process is repeated multiple times, yielding millions of copies of the desired DNA segment.

Summary

The polymerase chain reaction (PCR) has revolutionized the field of molecular biology by enabling the amplification and detection of specific DNA sequences. The technique involves denaturing the double-stranded DNA, annealing primers to the DNA strands, and extending the primers using DNA polymerase enzymes. The process is repeated multiple times, yielding millions of copies of the desired DNA segment.

Activities

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

Exercise 3.3: Polymerase Chain Reaction

Objective: Students will learn the principles of PCR and how to perform the technique.

Materials:
- DNA sample
- PCR reagents
- Thermocycler

Procedure:
1. Set up the PCR reaction mixture according to the manufacturer's instructions.
2. Load the reaction mixture into the thermocycler.
3. Program the thermocycler with the following cycle parameters: denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute.
4. Incubate the reaction mixture in the thermocycler for the programmed cycles.
5. Run a gel to verify the product of the PCR reaction.

Assessment

Assessment rubrics help students understand what is expected of them and how to complete tasks with proficiency.

Chapter overview

A road map of subject matter covered in each chapter.

Four types of vignettes show how biotechnology concepts covered in the chapter play a role in our daily lives. Vignette topics include 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 to claim proficiency in those skills are described in the Laboratory Skills Assessment Rubric.

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

Background sections for each chapter include biological theory behind the techniques and descriptions of the techniques themselves.

Essay questions follow the background section for each chapter and act as starting points for independent literature research beyond the textbook.

Graphics illustrate the hands-on activities to help students learn techniques.
**Teacher Supplement: Step-by-step activity preparation**

**Activity 3.B Disk Diffusion Test (Modified Kirby-Bauer Test)**

**Disk Diffusion Test (Modified Kirby-Bauer Test)**

This inquiry-based activity teaches students aseptic technique and enables them to test the relative potencies of antimicrobial compounds. Students will use guided inquiry strategies to explore nanotechnology by testing the antimicrobial qualities of silver nanoparticles (colloids). Students will also test household antimicrobial products such as antibacterial hand soap containing tricladin. In this activity, students will perform a disk diffusion test (a modified Kirby-Bauer test). The Kirby-Bauer test is very precise method used in hospital laboratories to determine the sensitivity of bacteria to different types of antibiotics. This test itself and its results are standardized.

**Activity Summary**

Students will use a 100 mm LB agar plate into quadrants and spread an E. coli HB101 bacterial culture on the plate to generate a bacterial lawn. Four paper disks will be impregnated with antimicrobials and tested; a negative control will be used as a control. Students will test the antimicrobial activity of the silver nanoparticles, a second control with antibiotic ampicillin, a third disk with silver nanoparticles, and a fourth control with a household antimicrobial product. A disk will be placed in each quadrant and the plate will be incubated overnight. If the test compound has antimicrobial properties against E. coli HB101, a zone of inhibition will form around the disk where the bacteria have been unable to grow. The area will be measured and compared to the other compounds and controls.

**Safety**

Ensure that students use aseptic technique and dispose of microorganisms properly by sterilizing or sealing in 10% bleach. Individuals with allergies to antibiotics (including antibiotics in the penicillin family) should avoid contact with ampicillin. Students should wear appropriate PPE.

**Activity Timelines**

- **Prepare the activity/homework:** 30 min
- **Set up the experiment:** 45 min
- **Measure zones of inhibition and general conditions:** 30 min

**Stopping Points**

If necessary, disks can be impregnated with the test compound and left to dry with the plate dish lid closed for 1-2 h at room temperature. Once dry, the disks can be stored overnight at 4°C. The bacterial culture can be stored for up to 5 days at 4°C before use; however, the culture is best when used fresh. Once the experiment is complete, the plates with the bacterial colonies can be disposed in the bin and stored for 1 week at 4°C prior to analysis.

**Tips**

- Students can guide the activity by choosing the compounds to test, however, review the compounds to ensure that they are not dangerous. In particular, ampicillin or penicillin allergies do not interfere with antibiotics.

**Anticipated Results**

When zones will form around each disk that has inhibited bacterial growth, these zones are referred to as zones of inhibition. Unlike the negative control, the ampicillin-impregnated disk should have a zone of inhibition. Occasionally, colonies will grow in the clear zone surrounding the antibiotic disk. These are antibiotic-resistant colonies that are either contaminants or have developed resistance to the antibiotics. The inhibition on the test disk will depend on the properties of the compound's uses. Technical inhibition of bacterial growth will yield clear zones.

**Analysis of Results**

Students should use a ruler to measure the inhibition for each of the compounds tested, sketch the results and organize the data for discussion with students about the lab data that will help them learn this skill, better predict results.

**Assessment**

Assign students formally or informally, and examine them, if they are eliminating compounds being tested and the rest of the student group. Similarly, have an explanation of the result results and improve the experiment or what is the next step of using the diffusion method. Some performed on students' ability to follow and record the data generated.

**Activity timelines and stopping points** help you plan activities.

**Instructional Videos and Presentations:**

Free online resources provide extra classroom guidance and assist with instruction.

**Inventory Files**

This is not a real activity; however, the Microbe and Health Kit and G20B Bacterial Transformation Kit contain these listed E. coli HB101 derivatives, LB agar powder, LB agar plates, snap caps, and ampicillin. This inventory serves 32 students working in groups of 4.

**Student-centered inquiry approach guidelines** help you get students to ask their own questions during activities.

**The teacher supplement is a full-size bound book with more than 200 pages to help you prepare and teach the activities in the student textbook.**
Activity 6.B GMO Detection by PCR

Activity Protocol

Part 1: Extracting Template DNA

1. Label one screwcap tube containing InstaGene™ matrix Non-GM and the other Test.

2. Weigh 0.5–2 g of non-GM control food and put it into the mortar. Record the mass.

3. Add 5 ml of dH₂O for every gram of food. To calculate the volume of water needed, multiply the mass (in grams) of the food by 5, and add that many milliliters of water.

   Mass of food = ___ g x 5 = ___ ml

4. Grind the food with the pestle for at least 2 min to form a slurry.

5. Add another 5 ml of dH₂O for every gram of food. Mix or grind further with the pestle until the slurry is smooth enough to pipet.

6. Use the graduated transfer pipet to transfer 50 μl of ground slurry to the screwcap tube labeled Non-GM. Recap the tube and shake or vortex to mix. This is the non-GM template DNA.

7. Wash the mortar and pestle with soap, wipe them with 10% bleach, rinse them well with tap water, and do a final rinse with dH₂O.

8. Repeat steps 2–5 with the test food to prepare the test food sample. Use the graduated transfer pipet to transfer 50 μl of the test food slurry to the screwcap tube labeled Test. Recap the tube and shake or vortex to mix. This is the test template DNA.

9. Incubate the Non-GM and Test screwcap tubes at 95°C for 5 min.

10. Place the tubes in a centrifuge in a balanced configuration and centrifuge for 5 min at maximum speed.

   Note: If using a mini centrifuge that can reach only 2,000 x g, centrifuge for 10 min.

11. Proceed directly to part 2 or store the gDNA in the screwcap tubes at 4°C for up to 1 month. Do not freeze the samples.
Work is being done to develop new antibiotics in order to strengthen the anti-microbial pipeline. Also, resources are being provided to support proper use of antibiotics in developing countries in order to slow the spread and development of antibiotic resistance.

3.2 Bacteria

Bacteria are much less complex than eukaryotic cells. The inside of the cell is referred to as the cytoplasm and contains 70S ribosomes used in protein synthesis during translation. Most bacteria contain a single loop of genomic DNA (gDNA) in a centralized area called a nucleoid and sometimes have small extra loops of DNA called plasmids, which are discussed in Chapter 5 and are important tools in genetic engineering.

Bacteria are enclosed by a plasma membrane and a peptidoglycan cell wall (see Figure 3.5). The cell wall is composed of two alternating sugars, N-acetylglucosamine (NAM) and N-acetylmuramic acid (NAG), in a polymer that is cross-linked with small peptides. Penicillin-based antibiotics prevent bacterial growth by disrupting the formation of the bacterial cell wall by inhibiting peptide cross-linking, which makes the cell wall weak. Bacteria are classified into two groups based on the thickness of their peptidoglycan cell wall (see Figure 3.6). Thick cell walls enable bacteria to absorb a microbiological stain called Gram stain, while thin walls cannot retain the stain. Bacteria are classified as gram-positive if they take up the stain or gram-negative if they do not. Bacteria may also be enclosed in a protective polysaccharide capsule that lies outside the cell wall.

Small hair-like projections called pili are often present on the outside of bacteria and help with cell-cell contact and adhesion. Many bacteria also have flagella that enable them to move and swim in aquatic environments.

Names and Shapes of Bacteria

Bacteria are named and classified by their shape. There are three major shapes of bacteria: coccus, bacillus, and spirillum. Cocci bacteria are spherical in shape and look like small balls under a microscope. Bacilli bacteria are oval or rod shaped and look like hot dogs. Spiroplasms are spiral-shaped (see Figure 3.7). The way bacteria arrange themselves when growing is also used in their names. For example, the prefix “strepto” comes from the Greek word streptos, which means twisted chain. When used in combination with the shape of a bacterium; for example, streptococcus, the name describes what the bacterium looks like. The term “staphylo” in staphylococcus comes from the Greek word staphyle, which means a bunch of grapes.

Bacterial Environments

Bacteria have many unique requirements for growth, including oxygen, temperature, and salt levels. Aerobic bacteria prefer high levels of oxygen to grow at their maximum rate. Anaerobic bacteria are called obligate aerobes if oxygen is absolutely necessary for their growth or facultative aerobes if they just grow better in the presence of oxygen but it is not required. Anaerobic bacteria prefer to grow in the absence of oxygen. Anaerobic bacteria are called obligate...
New in the Second Edition

Biotechnology: A Laboratory Skills Course, second edition, by J. Kirk Brown integrates concepts and hands-on laboratory activities together with real-world applications for your biotechnology course.

- Updated science content, including 10 new advanced topics and a new DNA barcoding activity — plus more background on industrial biotechnology practices and drug development
- New teacher supports to infuse more student-directed inquiry into laboratory activities
- Over 30 new and updated vignettes spanning careers in biotech, bioethical issues, and biotechnology in the real world
- Now aligned to the Biotechnology Assistant Credentialing Exam from Biotility

Now available

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