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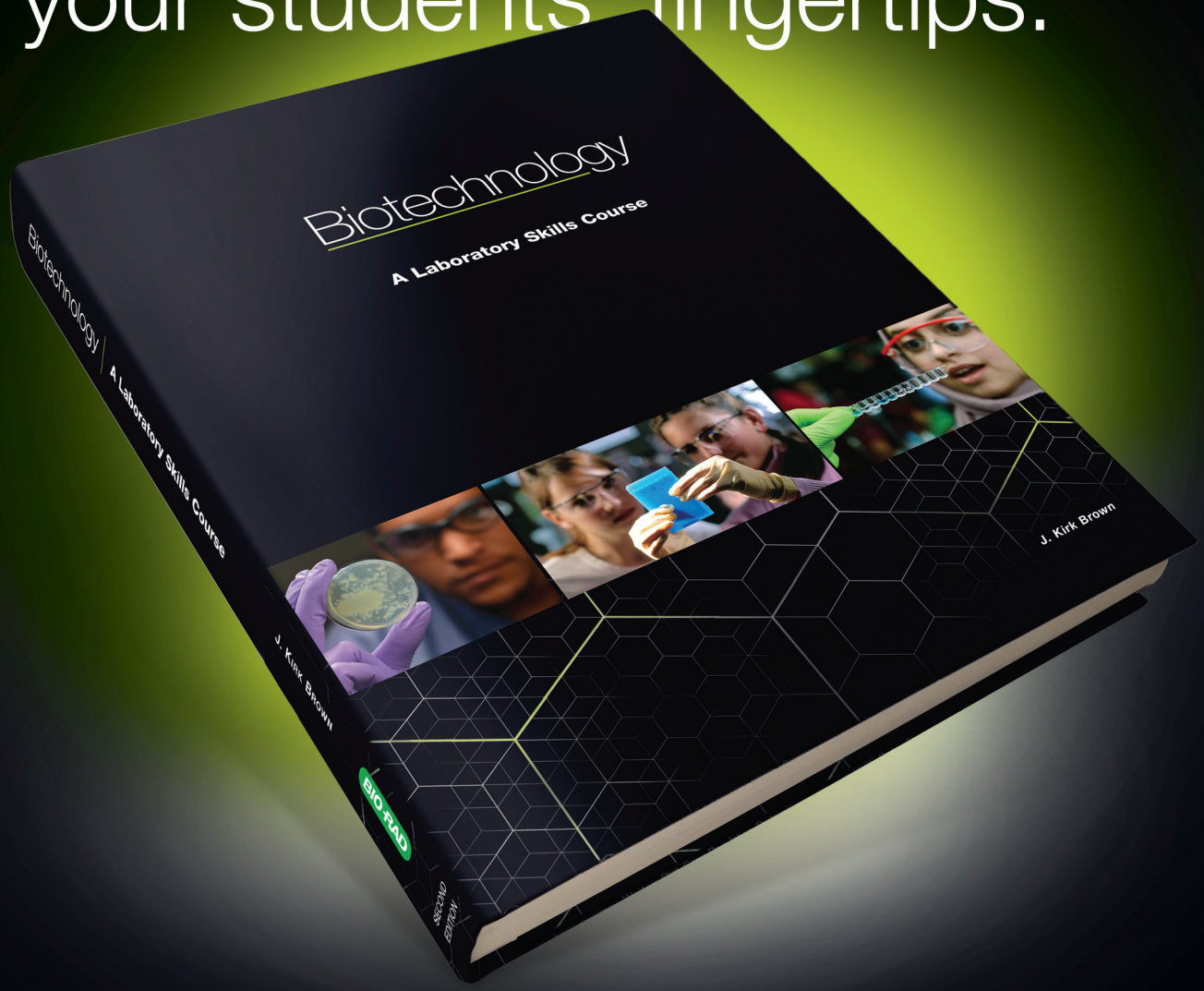
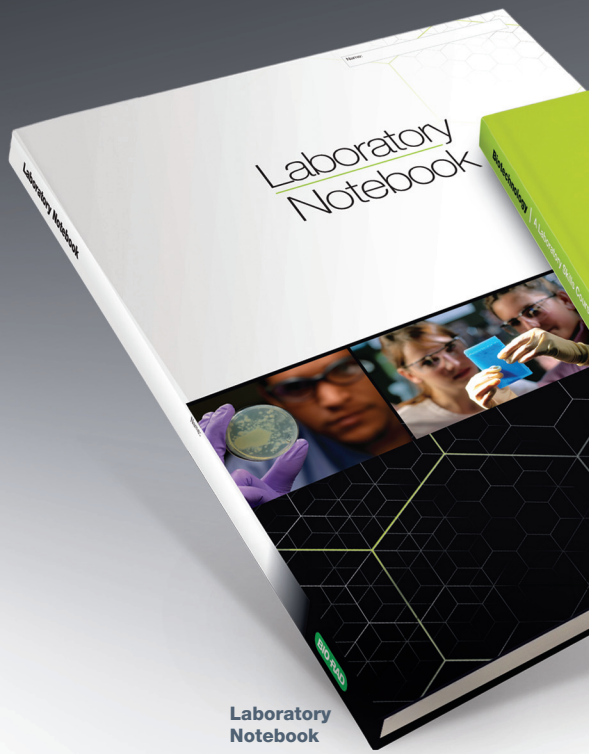


Biotechnology:
A Laboratory Skills Course
Second Edition

BIO-RAD

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.



Laboratory Notebook

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.

Biotility™
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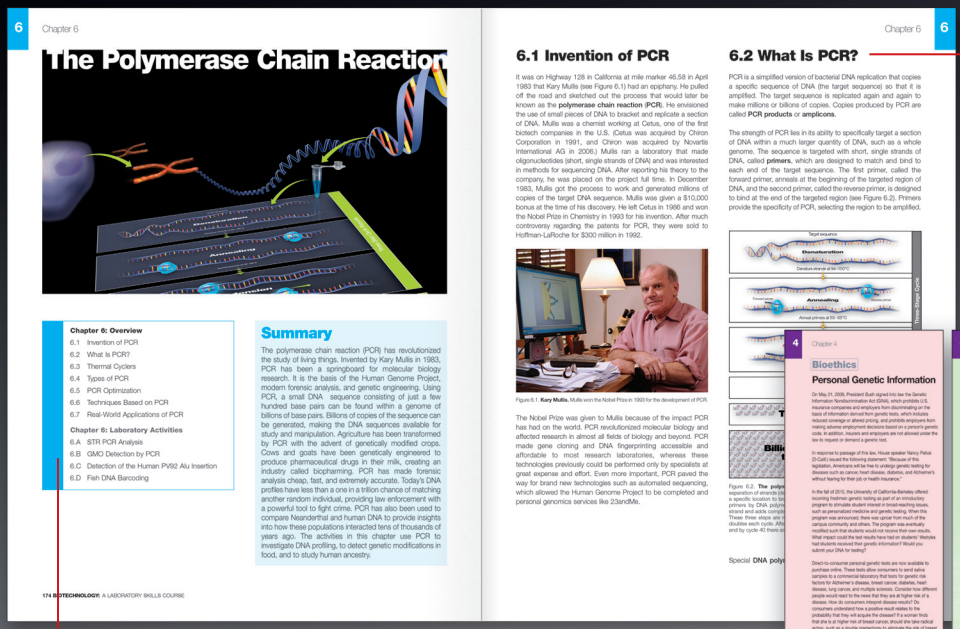
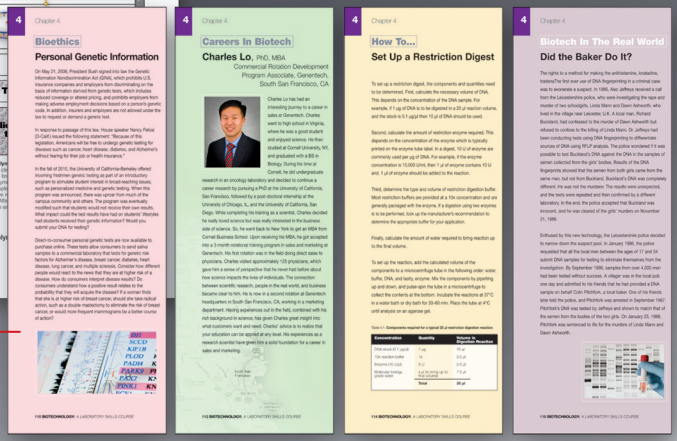
Student Textbook: A Complete Biotechnology Experience





Chapter overview gives 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.

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

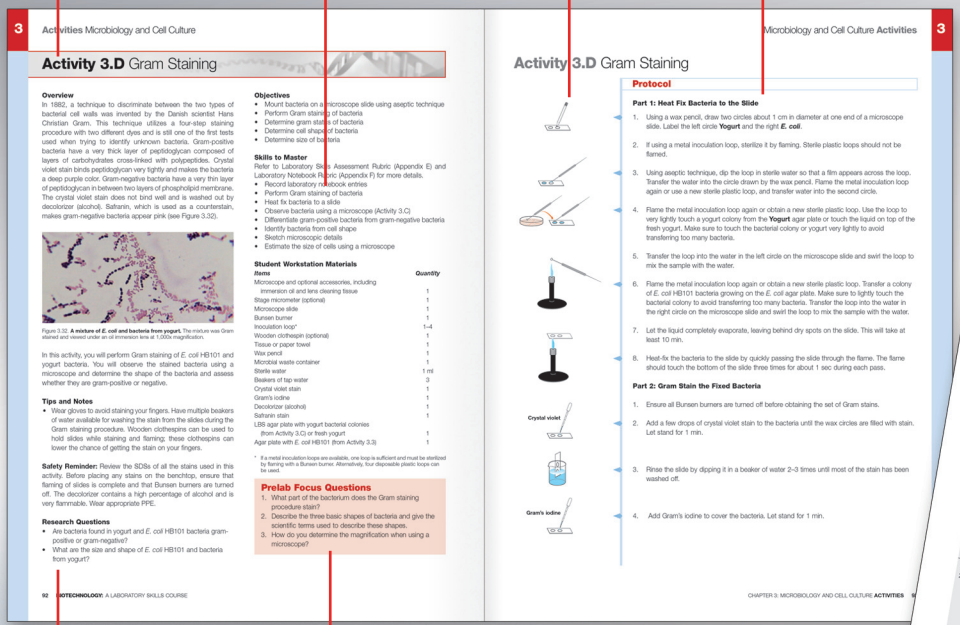
Essay questions follow the background section for each chapter and act as starting points for independent literature research beyond the textbook.

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.

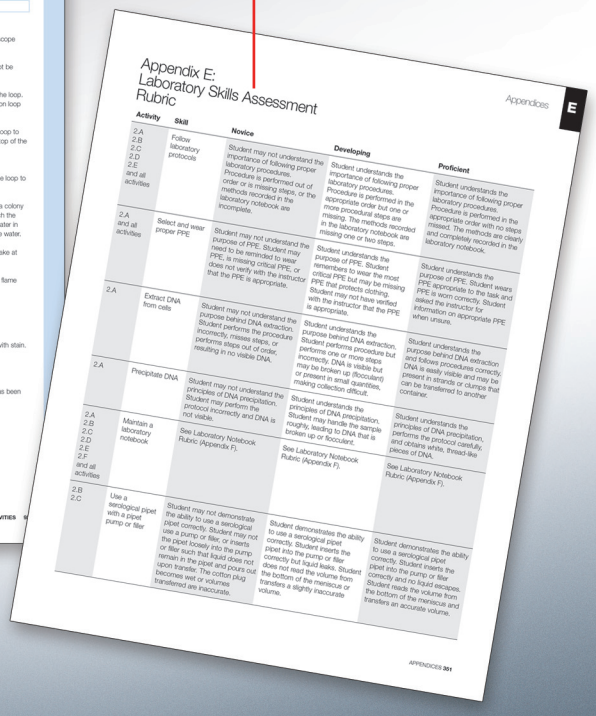
Laboratory skills are acquired by performing the activity. The requirements to claim proficiency in those skills are described in the Laboratory Skills Assessment Rubric.

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.



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



Research questions and objectives outline the experiments.

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

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 properties 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 trichlosan. In this activity, students will perform a disk diffusion test (a modified Kirby-Bauer test). The Kirby-Bauer test is a very precise method used in hospital laboratories to determine the sensitivity of bacteria to different types of antibiotics. The test itself and its results are standardized.

Activity Summary
Students will divide 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 with no antimicrobial agent, a positive control with the antibiotic ampicillin, a test disk with silver nanoparticles, and a second test disk 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. This area can be measured and compared to the other compounds and controls.

Students require one 100 mm LB agar plate poured in Activity 3.A.

Safety
Ensure that students use aseptic technique and dispose of microorganisms properly by autoclaving or soaking 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 Timeline

Tasks	Time	Notes/Reminders
Review the activity/lecture	30 min	Review the disk diffusion method.
Set up the experiment	45 min	Waiting for the disks to dry may create some dead time. An overnight incubation is required.
Measure zones of inhibition and generate conclusions	30 min	

Stopping Points
If necessary, disks can be impregnated with the test compounds and left to dry with the petri dish lid closed for 1–2 hr 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 wrapped in Parafilm and stored for 1 week at 4°C prior to analysis.

Tips
- Students can guide this activity by choosing the compounds to test. However, review the compounds to ensure that they are not hazardous. In particular, ensure students with ampicillin/penicillin allergies do not investigate antibiotics.

Anticipated Results
Clear 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 mutan[illegible]teria that have developed resistance to the antibiotics. The p[illegible] inhibition on the test disks will depend [illegible] properties of the compounds used. Trichlose[illegible] inhibitor of bacterial growth, while silver colloid[illegible] potent.

Analysis of Results
Students should use a ruler to measure the[illegible] inhibition for each of the compounds teste[illegible] sketch the results and organize the data in[illegible] a discussion with students about the be[illegible] data will help them learn this skill; tables [illegible] effectively to record data.

Assessment
Assess students formatively by obser[illegible] and ensuring that they are employing[illegible] compounds being tested and the ex[illegible] student groups. Similarly, have an[illegible] implication of the final results and [illegible] improve the experiment or what oth[illegible] using the diffusion method. Sum[illegible] performed on students' ability to g[illegible] how well students organize the da[illegible]

Activity timelines and stopping points help you plan 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 summaries**
- **Laboratory preparation steps**
- **Tips**
- **Safety**
- **Anticipated results**
- **Answers to pre- and postlab questions**
- **Assessment ideas**

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

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

Student-centered Inquiry Approach Guidelines

Teacher does	Student does
Engaging Phenomenon	1. Display images of Kirby Bauer test results on a bacterial plate.
	2. Write what is going on around each of the disks on the bacterial plate. 3. Students discuss their thinking with a partner and come up with a shared understanding of what they think is going on.
Model and/or acquire more information	4. Read a short passage about how the Kirby Bauer test works. 5. Go back to original understanding and update/modify if appropriate.
Explore/explain	6. Discuss testable and untestable questions
	7. Generate scientific questions that could be explored using the Kirby Bauer test. 8. Write questions on strips or sticky notes and place them on the white/chalkboard. 9. Class sorts the questions as testable or untestable questions.
Elaborate/evaluate	10. Engage in the activity in the book. 11. Design a modified version of the activity to investigate.

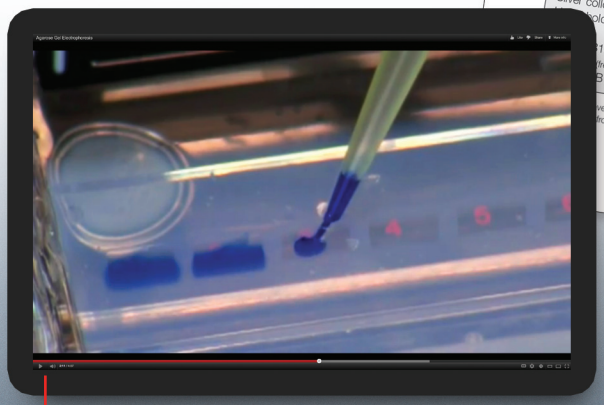
Inventory Table
This is not a kit based activity; however, the Microbes and Health kit and pGLO Bacterial Transformation kit contain freeze-dried *E. coli* HB101 bacteria, LB agar powder, LB agar broth capsules, and ampicillin. This inventory serves 32 students working in groups of 4.

Item	Source	Quantity per Class	Quantity per Team
Incubator	Instructor	1	1 (shared)
Autoclave and autoclave tape (optional)*	Instructor	1	N/A
2–20 µl adjustable-volume micropipets and sterile tips	Instructor	8	1
100–1,000 µl adjustable-volume micropipets and sterile tips, or 1 ml sterile graduated transfer pipets	Instructor	8	1
Rulers	Instructor	8	1
Small beaker	Instructor	1	N/A
15 ml culture tubes	Instructor	8	1
Microcentrifuge tubes (for dispensing)	Instructor	25	3
Petri plates (to hold paper disks)	Instructor	8	1
Sterile paper disks**	Instructor	32	N/A
Bunsen burners	Instructor	8	1
Inoculation loops or bacterial spreaders	Instructor	8	1
Sterile forceps	Instructor	8	1
Laboratory marking pens	Instructor	8	1
Pencils	Instructor	8	1
Microbial waste containers	Instructor	8	1
Silver colloid solution	Instructor	160 µl	20 µl
[illegible]old antimicrobial product	Instructor/Activity 3.1	160 µl	20 µl
[illegible]101 bacteria (freeze-dried or colonies on agar plate)	Instructor/kit	16 ml	2 ml
(freeze-dried vial or 1 mg/ml solution)	Instructor/kit	1 vial	2 ml of final culture
[illegible]B agar plates	Instructor/Activity 3.1	160 µl	20 µl
		8	1

[illegible]ve is not available, see the activity summary for information on using a microwave oven or hot plate to make media.
[illegible]rom Whatman #1 filter paper using a hole punch can be used. Be sure to autoclave them on a dry cycle for 15–20 minutes.

Inventory lists are available for every activity.

Instructional Videos and Presentations:
Free online resources support implementation

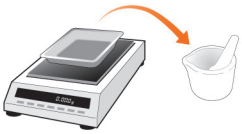


Supplementary technique videos and PowerPoint files provide extra classroom guidance and assist with instruction.

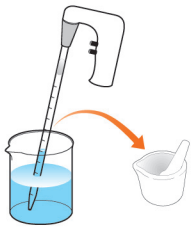
Activity 6.B GMO Detection by PCR

Activity Protocol

Part 1: Extracting Template DNA



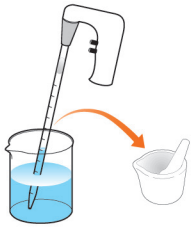
1. Label one screwcap tube containing InstaGene™ matrix **Non-GM** and the other **Test**.
2. Weigh 0.5–2 g of non-GM control food and put it into the mortar. Record the mass.



3. Add 5 ml of dH_2O 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.

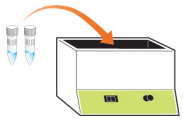
   Mass of food = ____g x 5 = _____ml

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



5. Add another 5 ml of dH_2O 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 dH_2O.
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.**

Example activity protocol page from student textbook

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-acetylmuramic acid (NAM) and N-acetylglucosamine (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.

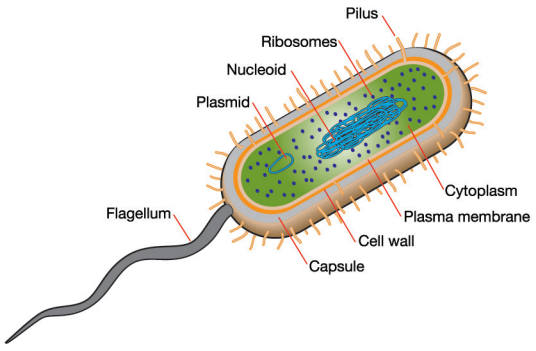


Figure 3.5. **Bacterial cell**. Bacteria have a plasma membrane, cell wall, nucleoid, and ribosomes. Many also have pili and flagella for locomotion, and some have plasmids.

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. Spirilli bacteria 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 *staphule*, which means a bunch of grapes.

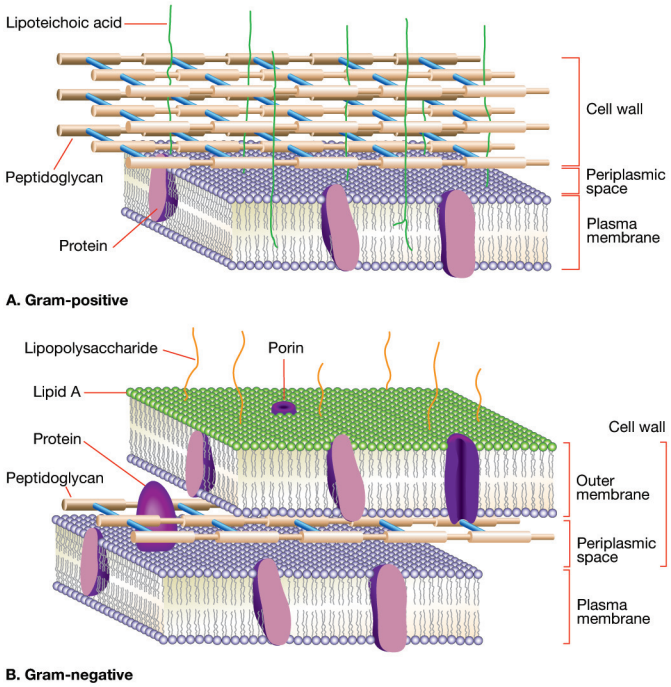


Figure 3.6. **Bacterial cell walls**. Gram-positive and gram-negative bacteria have cell walls of different thicknesses due to differences in their peptidoglycan content.

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. Aerobic 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

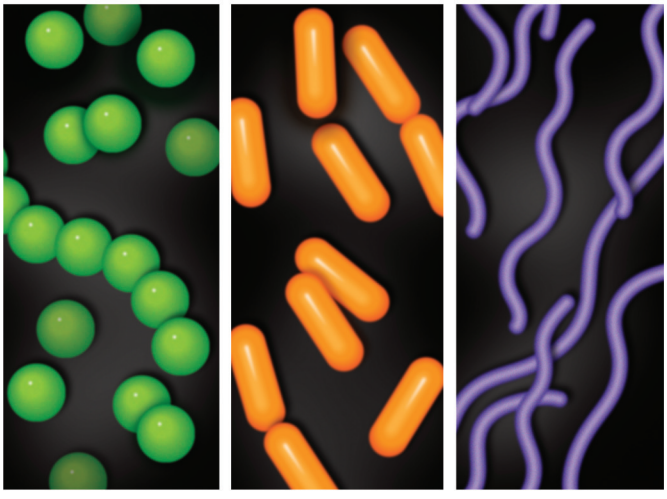


Figure 3.7. **Bacterial shapes**. Graphical rendering of three common types of bacterial morphology. From left to right: coccus, bacilus, and spirillum bacteria.

Example background page from student textbook

New in the Second Edition

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12008528EDU	**Student edition**	123.75	99.00
12008527EDU	**Teacher Supplement**	43.75	35.00
1661051EDU	**Laboratory Notebook**	18.75	15.00
1661053EDU	**Laboratory Notebook**, classroom set of 32	600.00	480.00

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