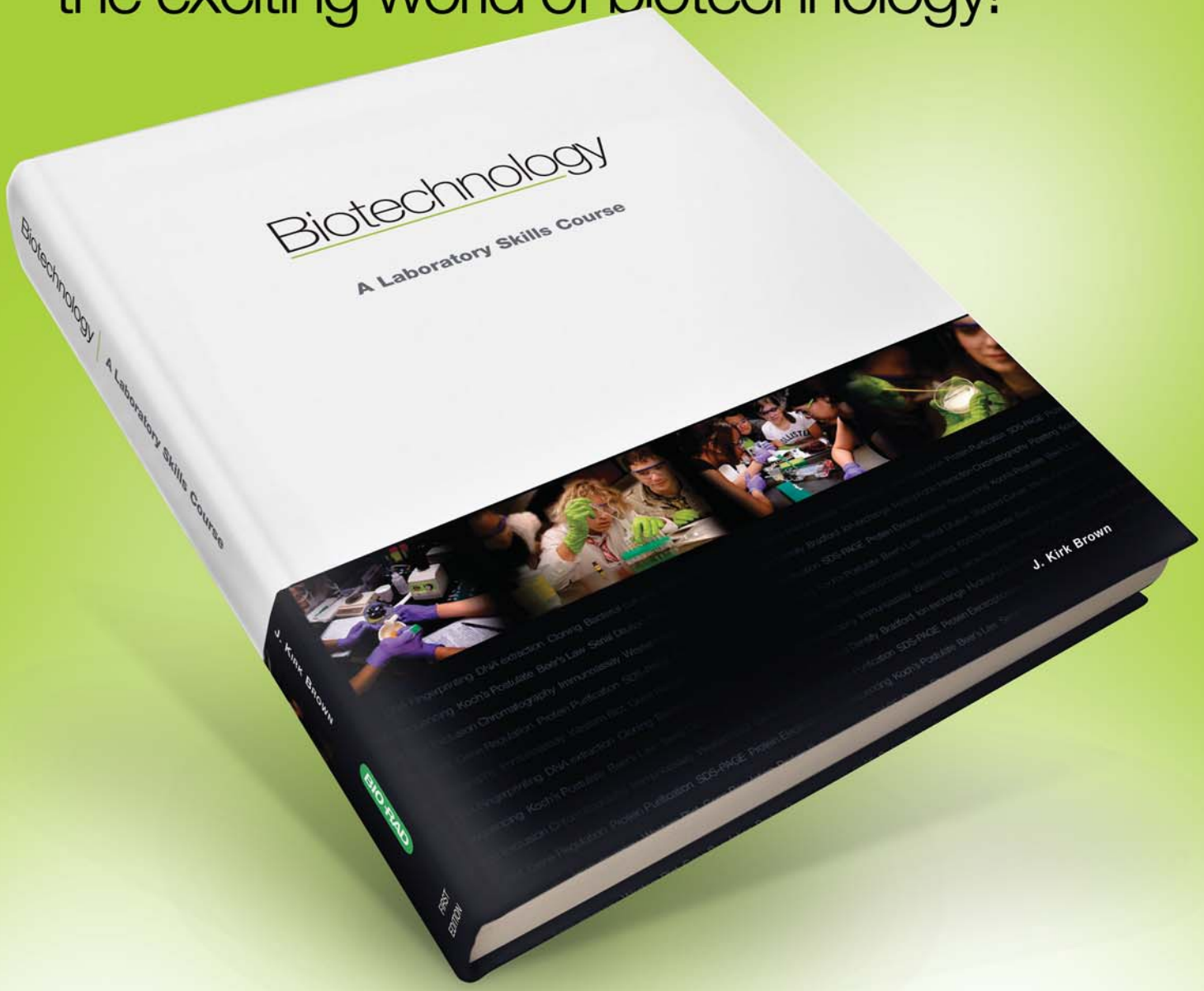
Biotechnology:
A Laboratory Skills Course
will guide you and your students through the exciting world of biotechnology!



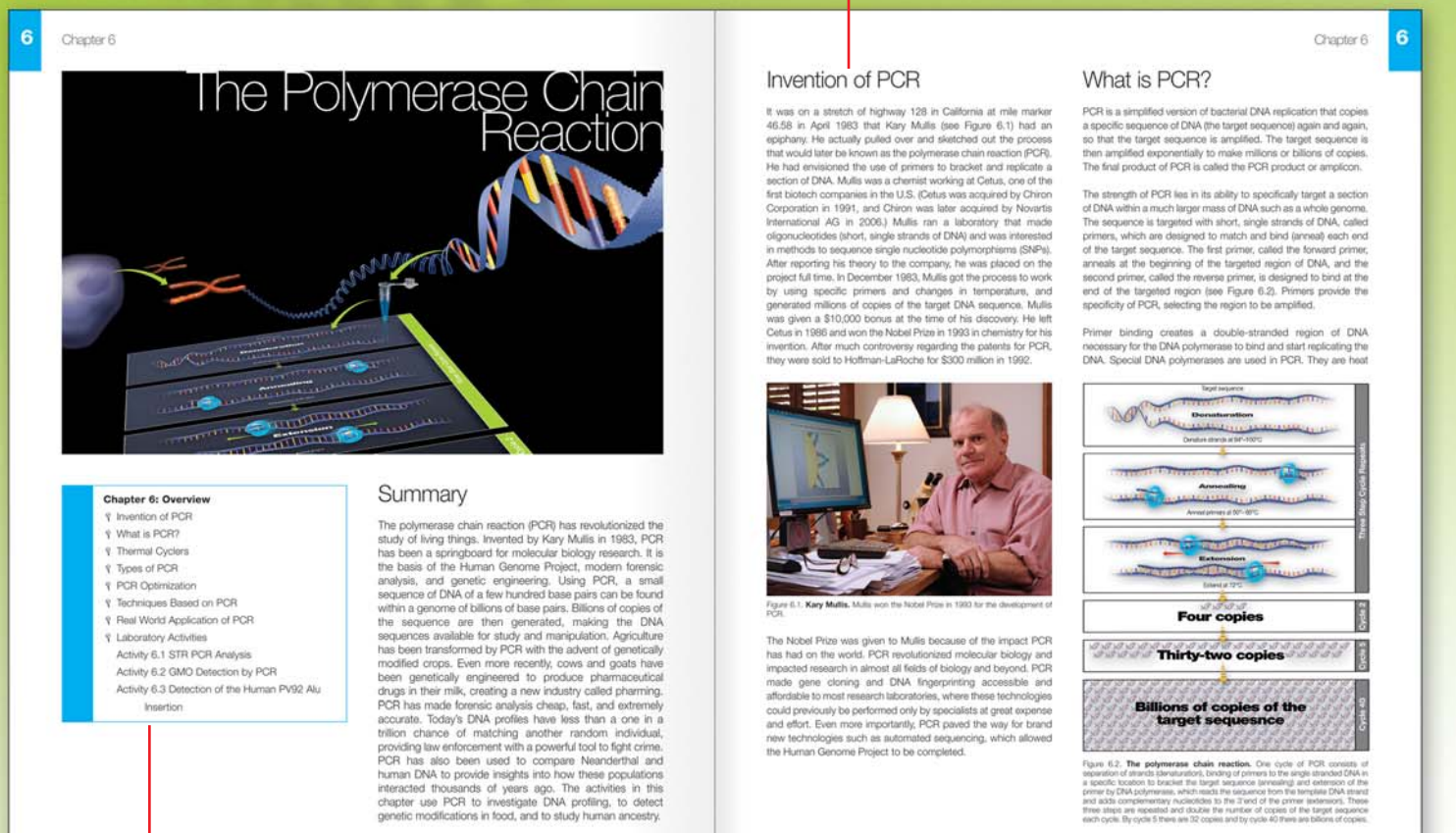
BIO-RAD

Student Textbook:
Content and Activity Sections

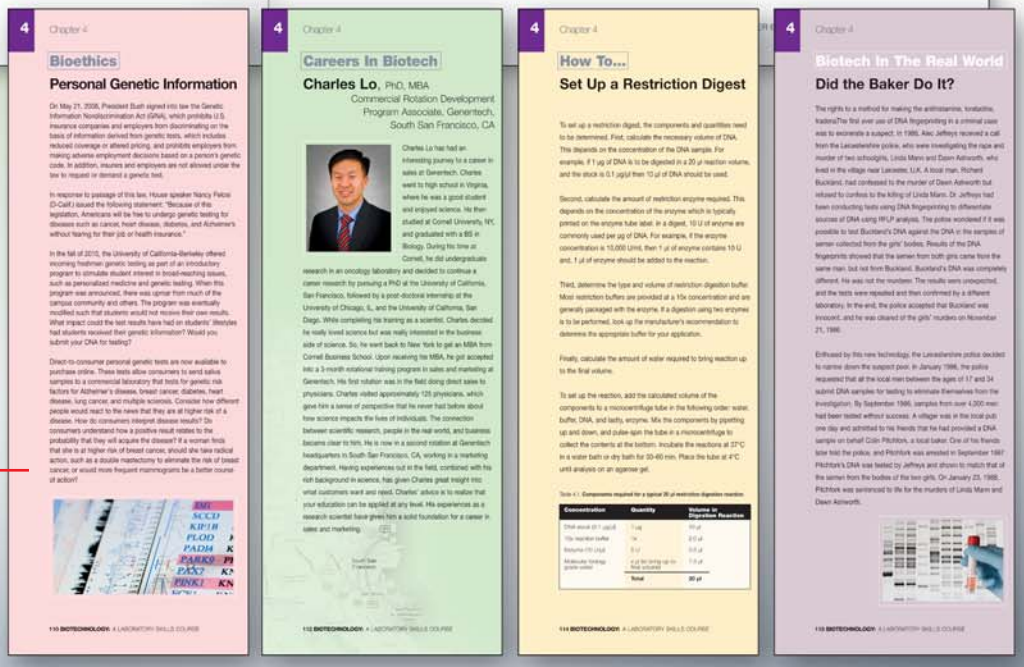
Student Textbook Content

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.



Chapter overview gives a roadmap of subject matter covered in the book.



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.

Student Textbook Vignettes

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 1882, a technique to discriminate between the two types of bacterial cell walls was invented by the Danish scientist Hans Christian Gram. This technique utilizes a four-step staining procedure using two different dyes and is still one of the first tests used when trying to identify unknown bacteria. Gram-positive bacteria have a very thick layer of peptidoglycan composed of layers of carbohydrates cross-linked with polypeptides. Crystal violet stain binds peptidoglycan very tightly and makes the bacteria a deep purple color. Gram-negative bacteria have a very thin layer of peptidoglycan in between two layers of phospholipid membrane, and the crystal violet stain does not bind well and is washed out by decolorizer (alcohol). Safranin, which is used as a counterstain, makes gram-negative bacteria appear pink (see Figure 3.32).

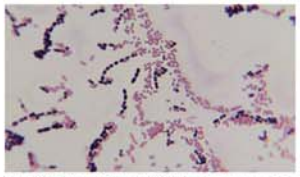


Figure 3.32 **A mixture of *E. coli* and bacteria from yogurt.** The mixture was Gram stained and viewed under an oil immersion lens at 1,000x magnification.

In this activity, you will perform Gram staining of *E. coli* HB101 and yogurt bacteria. You will observe the stained bacteria using a microscope and determine the shape of the bacteria and assess whether they are gram-positive or negative.

Tips and Notes

Wear gloves to avoid staining your fingers. Have multiple beakers of water available for washing the stain from the slides during the Gram staining procedure. Wooden clothes-pegs can be used to hold slides while staining and flaming; these clothes-pegs can lower the chance of getting the stain on your fingers.

Safety Reminder: Review the MSDS of all the stains used in this activity. Before placing any stains on the benchtop, ensure that flaming of slides is complete and Bunsen burners are turned off. The decolorizer contains a high percentage of alcohol and is very flammable. Wear appropriate PPE.

Research Questions

- Are bacteria found in yogurt and *E. coli* HB101 bacteria gram-positive or gram-negative?
- What is the size and shape of *E. coli* HB101 and bacteria from yogurt?

Objectives

- Mount bacteria on a microscope slide using aseptic technique
- Perform Gram staining of bacteria
- Determine gram status of bacteria
- Determine cell shape of bacteria
- Determine size of bacteria

Skills to Master

- Perform Gram staining of bacteria
- Heat fix bacteria to a slide
- Observe bacteria using a microscope
- Differentiate gram-positive bacteria from gram-negative bacteria
- Identify bacteria from cell shape
- Estimate the size of cells using a microscope

Student Workstation Materials

Items	*Quantity*
Microscope and optional accessories, including immersion oil and lens cleaning tissue	1
Stage micrometer (optional)	1
Microscope slide	1
Bunsen burner	1
Inoculation loop*	1–2
Wooden clothes peg (optional)	1
Tissue or paper towel	1
Wax pencil	1
Microbial waste container	1
Sterile water	1 ml
Beakers of tap water	3
Crystal violet stain	1
Gram's iodine	1
Decolorizer (alcohol)	1
Safranin stain	1
LBS agar plate with yogurt bacterial colonies (from Activity 3.3) or fresh yogurt	1
Agar plate with *E. coli* HB101 (from Activity 3.3)	1

* If a metal inoculation loops are available, one loop is sufficient and must be sterilized by flaming with a Bunsen burner. Alternatively, two disposable plastic loops can be used.

Prelab Focus Questions

1. **What part of the bacterium does the Gram staining procedure stain?**
2. **Describe the three basic shapes of bacteria, and give the scientific terms used to describe these shapes.**
3. **How do you determine the magnification when using a microscope?**

Activity 3.4 Gram Staining Protocol

Activity Protocol

Part 1: Heat Fix Bacteria to the Slide

1. Using a wax pencil, draw two circles about 1 cm in diameter at one end of a microscope slide. Label the left circle **Yogurt** and the right ***E. coli***.
2. If using a metal inoculation loop, sterilize it by flaming. Sterile plastic loops should not be flamed.
3. Using aseptic technique, dip the loop in sterile water so that a film appears across the loop. Transfer the water into the circle drawn by the wax pencil. Flame the metal inoculation loop again or use a new sterile plastic loop, and transfer water into the second circle.
4. Flame the metal inoculation loop again or obtain a new sterile plastic loop. Use the loop to very lightly touch a yogurt colony from the **yogurt** agar plate or touch the liquid on top of the fresh yogurt. Make sure to touch the bacterial colony or yogurt very lightly to avoid transferring too many bacteria.

Appendix E: Laboratory Skills Assessment Rubric

Activity	Skill	Novice	Developing	Proficient
2.1 2.2 2.3 2.4 2.5 and all activities	Follow laboratory protocols	Student may not understand the importance of following proper laboratory procedures. Procedure is performed out of order or is missing steps, or the methods recorded in the laboratory notebook are incomplete.	Student understands the importance of following proper laboratory procedures. Procedure is performed in the appropriate order but one or more procedural steps are missing. The methods recorded in the laboratory notebook are missing one or two steps.	Student understands the importance of following proper laboratory procedures. Procedure is performed in the appropriate order with no steps missed. The methods are clearly and completely recorded in the laboratory notebook.
2.1 and all activities	Select and wear proper PPE	Student may not understand the purpose of PPE. Student may need to be reminded to wear PPE, is missing critical PPE, or does not verify with the instructor that the PPE is appropriate.	Student understands the purpose of PPE. Student remembers to wear the most critical PPE but may be missing PPE that protects clothing. Student may not have verified with the instructor that the PPE is appropriate.	Student understands the purpose of PPE. Student wears PPE appropriate to the task and PPE is worn correctly. Student asked the instructor for information on appropriate PPE when unsure.
2.1	Extract DNA from cells	Student may not understand the purpose behind DNA extraction. Student performs the procedure incorrectly, misses steps, or performs steps out of order, resulting in no visible DNA.	Student understands the purpose behind DNA extraction. Student performs procedure but performs one or more steps incorrectly. DNA is visible but may be broken up (flocculant) or present in small quantities, making collection difficult.	Student understands the purpose behind DNA extraction and follows procedures correctly. DNA is easily visible and may be present in strands or clumps that can be transferred to another container.
2.1	Precipitate DNA	Student may not understand the principles of DNA precipitation. Student may perform the protocol incorrectly and DNA is not visible.	Student understands the principles of DNA precipitation. Student may handle the sample roughly, leading to DNA that is broken up or flocculent.	Student understands the principles of DNA precipitation, performs the protocol carefully, and obtains white, thread-like pieces of DNA.
2.1 2.2 2.3 2.4 2.5 2.6 and all activities	Maintain a laboratory notebook	See Laboratory Notebook Rubric (Appendix F).	See Laboratory Notebook Rubric (Appendix F).	See Laboratory Notebook Rubric (Appendix F).
2.2 2.3	Use a serological pipet with a pipet pump or filler	Student may not demonstrate the ability to use a serological pipet correctly. Student may not use a pump or filler, or inserts the pipet loosely into the pump or filler such that liquid does not remain in the pipet and pours out upon transfer. The cotton plug becomes wet or volumes transferred are inaccurate.	Student demonstrates the ability to use a serological pipet correctly. Student inserts the pipet into the pump or filler correctly but liquid leaks. Student does not read the volume from the bottom of the meniscus or transfers a slightly inaccurate volume.	Student demonstrates the ability to use a serological pipet correctly. Student inserts the pipet into the pump or filler correctly and no liquid escapes. Student reads the volume from the bottom of the meniscus and transfers an accurate volume.

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.

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

Student Textbook Activities and Assessment Rubics

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).

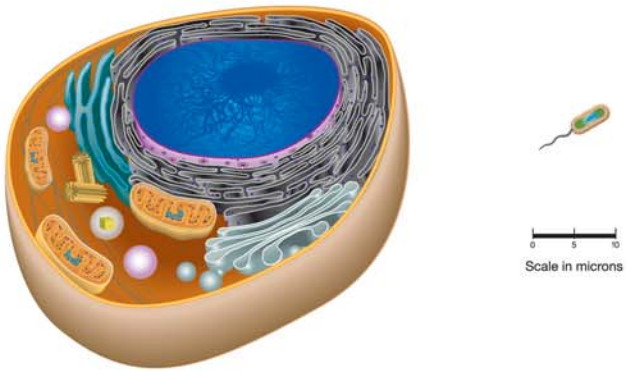


Figure 3.1. **Size comparison between prokaryotic and eukaryotic cells**.

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.

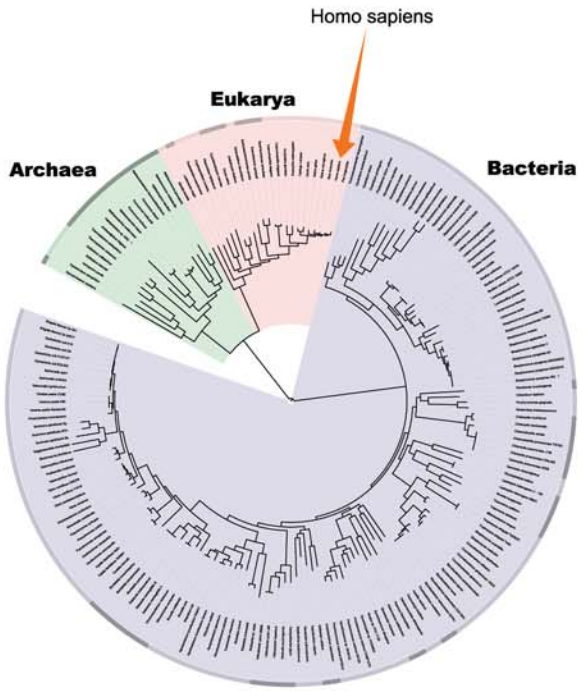


Figure 3.2. **The three domains of life**. This is a phylogenetic tree based on the genetic similarities between organisms that have had their genomes sequenced. Three domains can be seen: Archaea (in green), Bacteria (in blue), and Eukarya (in pink). The position of humans (*Homo sapiens*) is shown. This image was generated using Interactive Tree Of Life (iTOL), an online phylogenetic tree viewer. iTOL (2006) http://itol.embl.de/. Accessed October 2, 2010.

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 to 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

Excerpt of background section from student textbook

Figure 2.10. **Adjustable-volume micropipets.**

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.

$V_{Final} - V_{Initial} = V_{Delivered}$, 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.

Figure 2.11. **A burette.**

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.

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.

Figure 2.12. **Dial readout from adjustable-volume micropipets.** From left to right, 2–20 µl, 20–200 µl, and 200–1,000 µl micropipets reading 15.16 µl, 127.4 µl, and 758 µl, respectively.

Once the volume is set and a pipet tip is installed, depress the micropipet plunger. The micropipet stops naturally as the target volume is reached — this is called the first, or soft, stop (see Figure 2.13). Place the tip into the liquid, and gently release the plunger. Once the set volume is drawn into the tip, move the pipet to the desired location and depress the plunger to deliver the contents. Depressing the plunger further to the second, or hard, stop blows out any residual contents. Remove the tip from the liquid before releasing the plunger to avoid sucking the liquid back into the pipet. Discard the tip by ejecting.

Always watch the liquid going into and out of the pipet tip so that you will notice if the tip is loose or blocked. Never use the hard stop except to blow out contents.

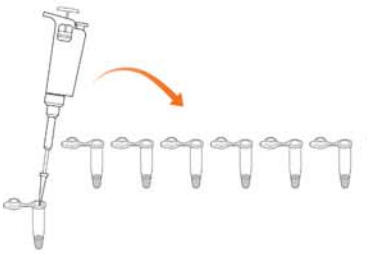
Figure 2.13. **Plunger positions of micropipets.** From left to right, plunger at rest, at soft stop, and at hard stop.

Excerpt of background section from student textbook with "How To..." vignette.

Activity 4.5 Forensic DNA Fingerprinting Protocol

Activity 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.

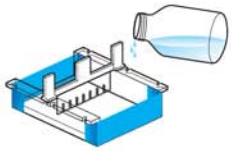
2. Using a fresh tip for each sample, pipet 10 µl of each DNA sample into the matching colored microcentrifuge tube. Make sure each sample is transferred to the bottom of the tube.

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.

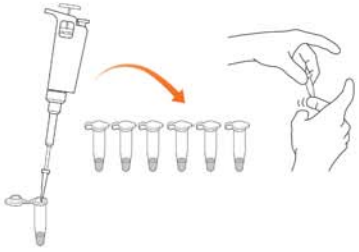
6. After the incubation, store the samples at 4°C until the next laboratory period. Samples can be stored for 1 month at 4°C. If there is sufficient time, proceed to running the gel.



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.

Excerpt of activity protocol from student textbook

Teacher Supplement:
Step-by-step activity preparation

The teacher supplement is a full-size bound book with more than 200 pages to help you prepare and teach the activities.


3 Teacher Supplement Microbiology and Cell Culture

Activity 3.5 Quantifying Bacterial Numbers

Quantifying Bacterial Numbers
In this activity, students will use aseptic technique, learn how to perform serial dilutions, and then use serial dilutions to quantify bacterial cell number. This activity uses an *E. coli* HB101 liquid culture, but the same technique could be used with yogurt. Each sample is diluted by a factor of 10; therefore, the culture is diluted 10x, 100x, 1,000x, and so on. (The amount that the culture is diluted by is called the dilution factor.) Another way to describe the dilution is that the sample is one-tenth, one-hundredth, and one-thousandth the concentration of the original culture. Students can conceptualize the dilution factor as a decimal point moving to the right. (If students consider the change to the concentration rather than dilution, the decimal point would move one place to the left.) This is great opportunity to revisit scientific notation.

Activity Summary
In this activity, students will make seven tenfold dilutions from a confluent *E. coli* HB101 liquid culture. In other words, the culture will be diluted 10,000,000 times, or by a factor of 10^7. A sample from each dilution will be spread onto LB agar plates. Each individual bacterium spread on the plate will grow into a colony and is referred to as a colony forming unit (CFU). Once grown, the number of CFU per plate will be determined. The number of bacteria per ml in the original culture will then be calculated by multiplying the number of CFU on the plate by the dilution factor.

Students require seven 60 mm LB agar plates poured in Activity 3.1.

Safety
Students should use aseptic technique and dispose of microorganisms properly by autoclaving or soaking in 10% bleach. Have a beaker of 10% bleach available at each workstation for disposal of contaminated pipet tips. Students should wear appropriate PPE.

Stopping Points
Serial dilutions must be spread on agar plates immediately since bacteria will continue to divide in the diluted samples. Once colonies have grown, the plates can be wrapped in Parafilm and stored at 4°C for up to a week until analysis can be performed.

Tips
Remind students to thoroughly mix or vortex samples before pipetting into the next dilution to ensure that the bacteria are evenly distributed.

Remind students to use an inoculation loop or bacterial spreader to spread the bacteria all over the plate; the streak plate technique should not be used for this activity.

Activity Timeline

Tasks	Time	Notes/Reminders
Review the activity/lecture	45 min	Review the concept of serial dilution and practice calculations from hypothetical results.
Perform serial dilutions and plate results	45 min	This activity requires an overnight incubation.
Count colonies and perform calculations	45 min	Encourage students to do the calculations by themselves.

Anticipated Results
Plates should show a tenfold decrease in the number of colonies formed across the dilution series. So if the last plate has 45 colonies, the previous plate should have approximately 450, and the plate before that should have around 4,500, and so on. The first three or four plates will likely have a bacterial lawn with too many colonies that are to count. The final three or four plates should be counted, and the last plate may have no colonies at all.

Analysis of Results
Students should begin by recording their results and counting the plates that have approximately 30–200 colonies. Plates with fewer than 30 colonies have large room for error. When plates have too many colonies to count, teach students to use the phrase "too many to count," or TMTC. When there are too many colonies to count, students should learn to differentiate a bacterial lawn (where individual colonies cannot be distinguished) from the scenario where individual colonies can be distinguished but there are just too many colonies to count. Students do not always see this distinction.

Once students have counted the number of CFU on the plate, they should calculate the number of bacteria in the original culture by multiplying the CFU by the dilution factor. Alternatively, the CFU can be divided by the concentration of the sample to calculate the number of bacteria in the original culture.

Students will also need to adjust for the fraction of bacteria plated, since only 100 µl of the 1 ml sample (that is, one-tenth of the total culture) was spread on the plate. Therefore, the actual number of CFU would have been 10 times higher if the entire 1 ml had been plated.

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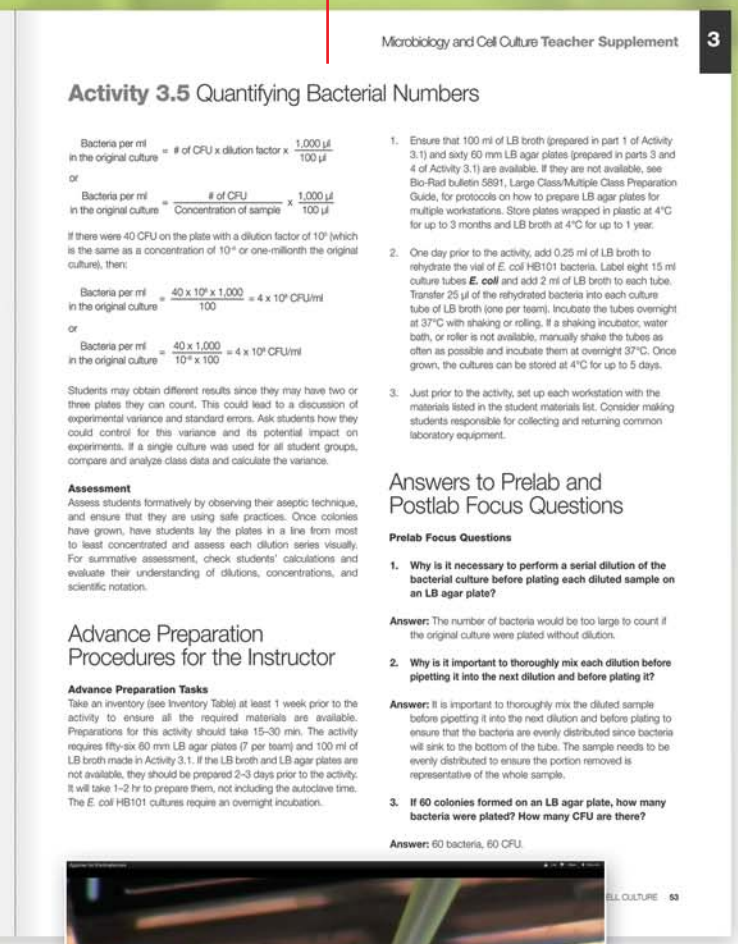

Microbiology and Cell Culture Teacher Supplement 3

Activity 3.5 Quantifying Bacterial Numbers

$$\text{Bacteria per ml in the original culture} = \#\text{ of CFU} \times \text{dilution factor} \times \frac{1{,}000\ \mu l}{100\ \mu l}$$

or

$$\text{Bacteria per ml in the original culture} = \frac{\#\text{ of CFU}}{\text{Concentration of sample}} \times \frac{1{,}000\ \mu l}{100\ \mu l}$$

If there were 40 CFU on the plate with a dilution factor of 10^5 (which is the same as a concentration of 10^{-5} or one-millionth the original culture), then:

$$\text{Bacteria per ml in the original culture} = \frac{40 \times 10^5 \times 1{,}000}{100} = 4 \times 10^8\ \text{CFU/ml}$$

or

$$\text{Bacteria per ml in the original culture} = \frac{40 \times 1{,}000}{10^{-5} \times 100} = 4 \times 10^8\ \text{CFU/ml}$$

Students may obtain different results since they may have two or three plates they can count. This could lead to a discussion of experimental variance and standard errors. Ask students how they could control for this variance and its potential impact on experiments. If a single culture was used for all student groups, compare and analyze class data and calculate the variance.

Assessment
Assess students formatively by observing their aseptic technique, and ensure that they are using safe practices. Once colonies have grown, have students lay the plates in a line from most to least concentrated and assess each dilution series visually. For summative assessment, check students' calculations and evaluate their understanding of dilutions, concentrations, and scientific notation.

Advance Preparation Procedures for the Instructor

Advance Preparation Tasks
Take an inventory (see Inventory Table) at least 1 week prior to the activity to ensure all the required materials are available. Preparations for this activity should take 15–30 min. The activity requires fifty-six 60 mm LB agar plates (7 per team) and 100 ml of LB broth made in Activity 3.1. If the LB broth and LB agar plates are not available, they should be prepared 2–3 days prior to the activity. It will take 1–2 hr to prepare them, not including the autoclave time. The *E. coli* HB101 cultures require an overnight incubation.

1. Ensure that 100 ml of LB broth (prepared in part 1 of Activity 3.1) and sixty 60 mm LB agar plates (prepared in parts 3 and 4 of Activity 3.1) are available. If they are not available, see Bio-Rad bulletin 5891, Large Class/Multiple Class Preparation Guide, for protocols on how to prepare LB agar plates for multiple workstations. Store plates wrapped in plastic at 4°C for up to 3 months and LB broth at 4°C for up to 1 year.
2. One day prior to the activity, add 0.25 ml of LB broth to rehydrate the vial of *E. coli* HB101 bacteria. Label eight 15 ml culture tubes ***E. coli*** and add 2 ml of LB broth to each tube. Transfer 25 µl of the rehydrated bacteria into each culture tube of LB broth (one per team). Incubate the tubes overnight at 37°C with shaking or rolling. If a shaking incubator, water bath, or roller is not available, manually shake the tubes as often as possible and incubate them at overnight 37°C. Once grown, the cultures can be stored at 4°C for up to 5 days.
3. Just prior to the activity, set up each workstation with the materials listed in the student materials list. Consider making students responsible for collecting and returning common laboratory equipment.

Answers to Prelab and Postlab Focus Questions

Prelab Focus Questions

1. **Why is it necessary to perform a serial dilution of the bacterial culture before plating each diluted sample on an LB agar plate?**

Answer: The number of bacteria would be too large to count if the original culture were plated without dilution.

2. **Why is it important to thoroughly mix each dilution before pipetting it into the next dilution and before plating it?**

Answer: It is important to thoroughly mix the diluted sample before pipetting it into the next dilution and before plating to ensure that the bacteria are evenly distributed since bacteria will sink to the bottom of the tube. The sample needs to be evenly distributed to ensure the portion removed is representative of the whole sample.

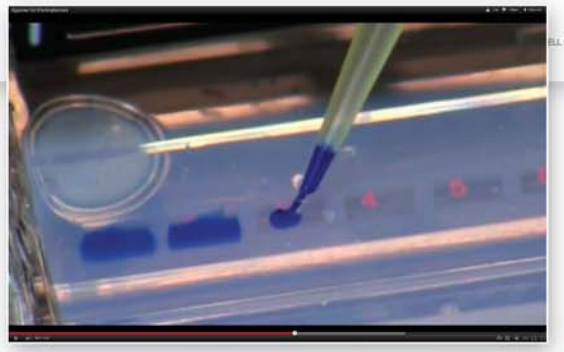
3. **If 60 colonies formed on an LB agar plate, how many bacteria were plated? How many CFU are there?**

Answer: 60 bacteria, 60 CFU.

ELL CULTURE 53

This supplement provides implementation guidance:

- Tips
- Safety
- Activity timelines
- Stopping points
- Anticipated and analysis of results
- Assessment
- Inventory table
- 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

Bringing Biotechnology to Your Classroom

The Textbook

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

Supplemental Materials

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.

The Author

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.

Ordering Information

Catalog #	Description
166-1025EDU	Biotechnology: A Laboratory Skills Course, Student Edition, ISBN 978-0-9832396-0-4
166-1027EDU	Biotechnology: A Laboratory Skills Course, Teacher Edition, includes 1 each Student Edition and Teacher Supplement (ISBN 978-0-9832396-1-1), and DVD set with technique videos and classroom presentations.
166-1051EDU	Laboratory Notebook, ISBN 978-0-9832396-2-8
166-1052EDU	Supplementary Materials DVD Set

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