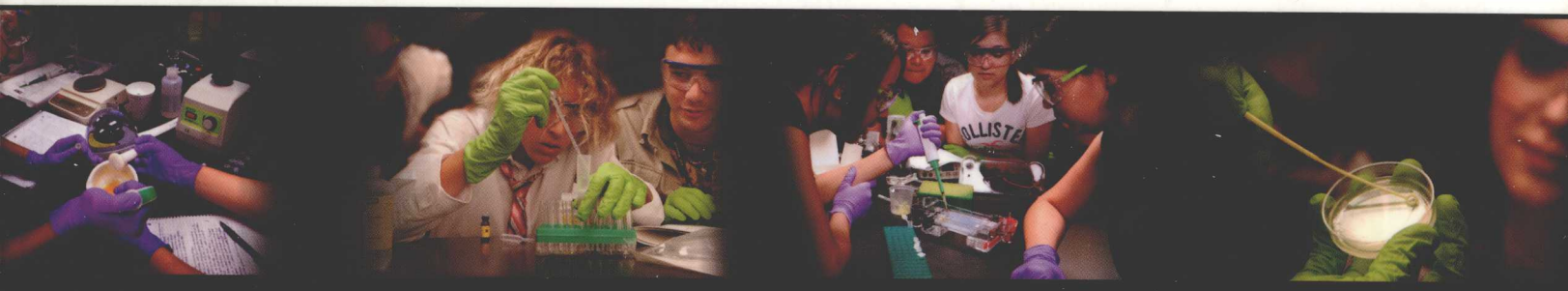
Biotechnology

A Laboratory Skills Course



J. Kirk Brown

Biotechnology

A Laboratory Skills Course

First Edition

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BIO-RAD

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Dedication

This book represents a lifetime of teaching experience and sacrifice. I would like to dedicate it to a number of people.

To my immediate family: my father and mother, Jim and Janice Brown, and my sister Lisa and her family, who fostered my natural curiosity into a lifetime of wonder about the world around me. To my wife, Lisa, and her family, whose love and support have always encouraged me to pursue my dreams and to grow, and to my daughter Lynae and son Ryan who mean everything to me, I dedicate this book.

To my Bio-Rad family, especially to my friend and colleague Stan Hitomi, to whom I am so very grateful for all of the adventures and collaboration. To my late friend Ron Mardigian for believing enough in two teachers to listen to our ideas about what teachers actually want and need to successfully teach biotechnology to their students. To Laurie Usinger for always believing in me and encouraging me to write this book. To Bryony Ruegg, whose continued support in editing my drafts and making suggestions in a manner that helped me stay on task and maintain focus, I owe so very much. To Ingrid, Julie, Erika, Michele, Sherri, Essy, Leigh, and all of the previous team members with whom I have traveled and taught with over the years, I dedicate this book.

To my Tracy Unified family, especially Dr Jim Franco and Dr Sheila Harrison, whose outside-the-box thinking has enabled me to live my life as a teacher in two worlds, one in the classroom and the other partially in the business world. To my colleagues in the science department, who are such wonderful teachers, and most important, to my former and current students for whom I continue to teach, I dedicate this book.

About the Author

J. Kirk Brown is a Nationally Board Certified Teacher and the Science Department Chair at Tracy High School, Tracy, CA, where he has taught for 23 years. He helps students develop into savvy biologists by integrating his teaching with inspiring hands-on laboratory experiences in his International Baccalaureate Biology courses and Biotechnology courses. Kirk also helped found the Agricultural/Scientific Academy at Tracy High School, which allows students to apply the science learned in the classroom to the community they live in.

Kirk's passion for education extends into and beyond the postsecondary level. As an adjunct associate professor at San Joaquin Delta College, Stockton, CA, he teaches courses in Core Biology and Fundamentals of Biotechnology, and as the lead instructor at the Edward Teller Education Center at the Lawrence Livermore National Laboratory (LLNL), Livermore, CA, he conducts teacher professional development programs. His collaborations with the California Department of Education, 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.

Kirk's dedication as a teacher has earned him numerous honors, recognition by students, and peer-nominated awards, including:

Genentech's Access Excellence Award (1996)
Milken National Educator Award (1999)
Outstanding Biology Teacher Award from the National Association of Biology Teachers (2003)
Carlston Family Foundation Outstanding Teachers of America Award (2006)
Biotechnology Educator DiNA Award from BayBio (inducted into the 2007 Pantheon)
Cortopassi Family Foundation Outstanding Science Teacher Award (2008)

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 plethora 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.

Preface

As a science educator, I have always found that the key to student engagement is providing relevance and context. Biotechnology, with its direct application to student lives—from disease treatment and environmental monitoring to the snack food they eat—resonates with and inspires students to learn.

As educators, we know that the best way to learn about science is to do science. *Biotechnology: A Laboratory Skills Course* was written to help students learn about biotechnology by performing laboratory activities. Whether or not students plan to pursue scientific careers, this course will give students an understanding of biotechnology and its implications in the real world that will serve them throughout their lives. Moreover, as my own students have experienced, the hands-on nature of and critical thinking required for biotechnology experiments help them develop college and career-ready skills that are necessary in a globally competitive world. The research skills gained in the course also raise students above their peers when pursuing careers in laboratory science and may also help students to secure part-time work in a laboratory while studying at college or university.

For me, it was logical to create a biotechnology textbook that incorporates Bio-Rad's Biotechnology Explorer™ kits. I have been working with Bio-Rad and incorporating their kits into my science courses for more than a decade. I have witnessed how teaching using quality kits that generate successful results builds student confidence and allows students to focus on mastering the skills and understanding the science. Once students have mastered the skills, they can then move on to more independent research and the challenges that come with it. Moreover, as a teacher, I find that using a kit based curriculum frees up my time so I can focus on teaching and developing my students. In addition, Bio-Rad's place as a leading provider of biotechnology tools means that students gain a competitive edge by training on similar equipment to that which they will use in university and industrial settings.

This book is divided into nine chapters that cover different biotechnological concepts. Each chapter contains a background section that introduces students to the biological theory behind the techniques and provides background to the techniques themselves. Chapters 2–8 include a series of activities that directly link to the chapter topic.

Chapter 1 is a general introduction to biotechnology, how it is used and regulated, and the types of careers available.

Chapter 2 focuses on basic skills that are essential to conducting biotechnology research, such as safety, maintaining a laboratory notebook, measuring volumes and mass, and making solutions.

Chapter 3 introduces microbiological and cell culturing techniques.

Chapters 4–6 introduce techniques for DNA manipulation, such as restriction digestion, transformation, and the polymerase chain reaction (PCR).

Chapter 7 covers basic protein analysis techniques, including protein quantitation, chromatography, and polyacrylamide gel electrophoresis (PAGE).

Chapter 8 extends concepts learned in Chapter 7 to the use of antibodies in protein research.

Chapter 9 is the culmination of the course, where students take the skills developed over the duration of the course and apply them to an independent research project.

The goal of this book is to provide sufficient background for the laboratory activities without being overwhelming. As such, the background and activities are included together in each chapter so that the relevant background information is located in the same place as the activities.

Activities are designed to progressively develop the laboratory skills and technical understanding that give students the grounding necessary to perform independent research. To this end, it is expected that laboratory notebooks are used for this course so they learn how to document experiments as actual research scientists do. Students should take responsibility for writing up experiments, analyzing results, and using critical thinking skills to generate conclusions. A Laboratory Notebook Rubric is included in Appendix F to help clarify the requirements for experimental writeups.

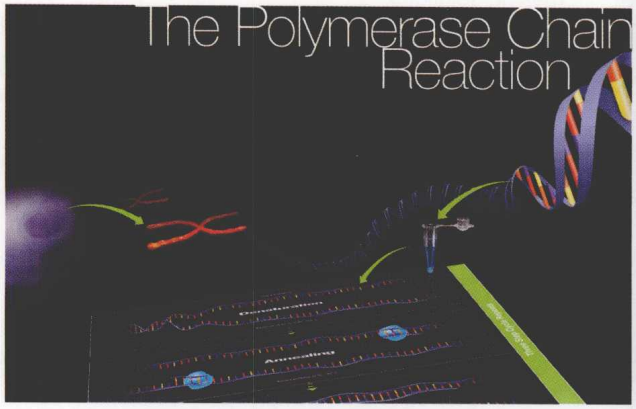
The activities in this book cross many subjects and can be used to supplement a wide variety of courses, from environmental and health science to genetics and cell biology. The activities also bring together science, technology, engineering, and mathematics (STEM) in an integrated set of tools and processes. Whether this book is used to integrate a few of these concepts or to build a complete course, students will gain valuable experience and a deeper appreciation and understanding of science and its role in the world today.

It is my hope that students are excited by this course and use the information and skills learned outside of the classroom. Biotechnology does not just happen in the laboratory; it affects our daily lives. This book connects laboratory techniques to life in the real world and should help students become biotech-savvy citizens better able to make informed decisions in their own lives and those of the people around them.

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.

The Polymerase Chain Reaction



Chapter 6: Overview

- Invention of PCR
- What Is PCR?
- Thermal Cyclers
- Types of PCR
- PCR Optimization
- Techniques Based on PCR
- Real-World Applications of PCR
- Laboratory Activities
  - Activity 6.1 STR PCR Analysis
  - Activity 6.2 GMO Detection by PCR
  - Activity 6.3 Detection of the Human PV92 Alu Insertion

Summary

The polymerase chain reaction (PCR) has revolutionized the study of living things. Invented by Kary Mullis in 1983, PCR has been a springboard for molecular biology research. It is the basis of the Human Genome Project, modern forensic analysis, and genetic engineering. Using PCR, a small DNA sequence consisting of just a few hundred base pairs can be found within a genome of billions of base pairs. Billions of copies of the sequence can be generated, making the DNA sequences available for study and manipulation. Agriculture has been transformed by PCR with the advent of genetically modified crops. Even more recently, cows and goats have been genetically engineered to produce pharmaceutical drugs in their milk, creating a new industry called pharming. PCR has made forensic analysis cheap, fast, and extremely accurate. Today's DNA profiles have less than a one in a trillion chance of matching another random individual, providing law enforcement with a powerful tool to fight crime. PCR has also been used to compare Neanderthal and human DNA to provide insights into how these populations interacted tens of thousands of years ago. The activities in this chapter use PCR to investigate DNA profiling, to detect genetic modifications in food, and to study human ancestry.

Invention of PCR

It was on Highway 128 in California at mile marker 46.58 in April 1983 that Kary Mullis (see Figure 6.1) had an epiphany. He pulled off the road and sketched out the process that would later be known as the **polymerase chain reaction (PCR)**. He envisioned the use of small pieces of DNA to bracket and replicate a section of DNA. Mullis was a chemist working at Cetus, one of the first biotech companies in the U.S. (Cetus was acquired by Chiron Corporation in 1991, and Chiron was acquired by Novartis International AG in 2006.) Mullis ran a laboratory that made oligonucleotides (short, single strands of DNA) and was interested in methods for sequencing DNA. After reporting his theory to the company, he was placed on the project full time. In December 1983, Mullis got the process to work and generated millions of copies of the target DNA sequence. Mullis was given a $10,000 bonus at the time of his discovery. He left Cetus in 1986 and won the Nobel Prize in Chemistry in 1993 for his invention. After much controversy regarding the patents for PCR, they were sold to Hoffman-LaRoche for $300 million in 1992.

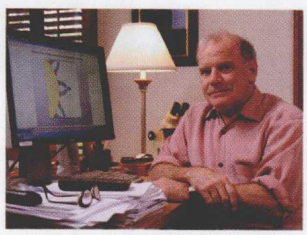


Figure 6.1. **Kary Mullis.** Mullis won the Nobel Prize in 1993 for the development of PCR.

The Nobel Prize was given to Mullis because of the impact PCR has had on the world. PCR revolutionized molecular biology and affected research in almost all fields of biology and beyond. PCR made gene cloning and DNA fingerprinting accessible and affordable to most research laboratories, whereas these technologies previously could be performed only by specialists at great expense and effort. Even more important, PCR paved the way for brand new technologies such as automated sequencing, which allowed the Human Genome Project to be completed.

What Is PCR?

PCR is a simplified version of bacterial DNA replication that copies a specific sequence of DNA (the target sequence) so that it is amplified. The target sequence is replicated again and again to make millions or billions of copies. Copies produced by PCR are called **PCR products** or **amplicons**.

The strength of PCR lies in its ability to specifically target a section of DNA within a much larger quantity of DNA, such as a whole genome. The sequence is targeted with short, single strands of DNA, called **primers**, which are designed to match and bind (**anneal**) each end of the target sequence. The first primer, called the forward primer, anneals at the beginning of the targeted region of DNA, and the second primer, called the reverse primer, is designed to bind at the end of the targeted region (see Figure 6.2). Primers provide the specificity of PCR, selecting the region to be amplified.

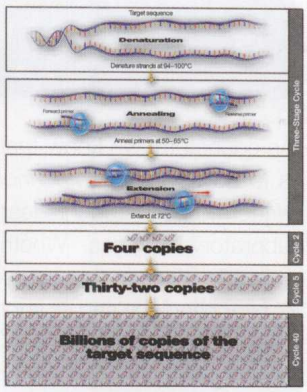
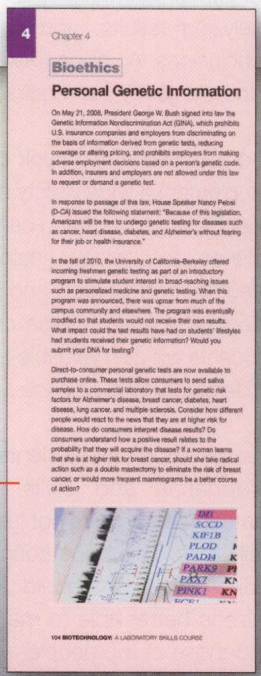


Figure 6.2. **The polymerase chain reaction.** One cycle of PCR consists of separation of strands (denaturation), binding of primers to the single-stranded DNA in a specific location to bracket the target sequence (annealing), and extension of the primers by DNA polymerase, which reads the sequence from the template DNA strand and adds complementary nucleotides to the 3′-end of the primers (extension). These three steps are repeated and the number of copies of the target sequence doubles each cycle. After cycle 2, there are 4 copies, by cycle 5 there are 32 copies, and by cycle 40 there are billions of copies.

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



Bioethics

Personal Genetic Information

On May 21, 2008, President George W. Bush signed into law the Genetic Information Nondiscrimination Act (GINA), which prohibits U.S. insurance companies and employers from discriminating on the basis of information derived from genetic tests, reducing coverage or altering pricing, and prohibits employers from making adverse employment decisions based on a person's genetic code. In addition, insurers and employers are not allowed under this law to request or demand a genetic test.

In response to passage of this law, House Speaker Nancy Pelosi (D-CA) issued the following statement: "Because of this legislation, Americans will be free to undergo genetic testing for diseases such as cancer, heart disease, diabetes, and Alzheimer's without fearing for their job or health insurance."

In the fall of 2010, the University of California–Berkeley offered incoming freshmen genetic testing as part of an introductory program to stimulate student interest in broad-reaching issues such as personalized medicine and genetic testing. When this program was announced, there was uproar from much of the campus community and elsewhere. The program was eventually modified so that students would not receive their own results. What impact could the test results have had on students' lifestyles had students received their genetic information? Would you submit your DNA for testing?

Direct-to-consumer personal genetic tests are now available to purchase online. These tests allow consumers to send saliva samples to a commercial laboratory that tests for genetic risk factors for Alzheimer's disease, breast cancer, diabetes, heart disease, lung cancer, and multiple sclerosis. Consider how different people would react to the news that they are at higher risk for disease. How do consumers interpret disease results? Do consumers understand how a positive result relates to the probability that they will acquire the disease? If a woman learns that she is at higher risk for breast cancer, should she take radical action such as a double mastectomy to eliminate the risk of breast cancer, or would more frequent mammograms be a better course of action?

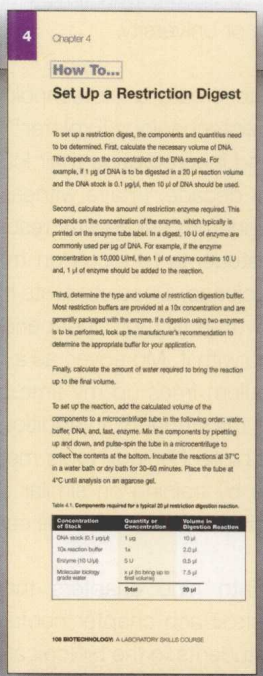
Careers In Biotech

Charles Lo, PhD, MBA

Commercial Rotation Development Program Associate, Genentech, South San Francisco, CA

Photo courtesy of Charles Lo

Charles Lo has had an interesting journey to a career in sales at Genentech. Charles went to high school in Virginia, where he was a good student and enjoyed science. He then studied at Cornell University, Ithaca, NY, and graduated with a BS in biology. During his time at Cornell, he did undergraduate research in an oncology laboratory and decided to continue a career in research by pursuing a PhD at the University of California–San Francisco, followed by post-doctoral internships at the University of Chicago, IL, and the University of California–San Diego. While completing his training as a scientist, Charles decided that he really loved science but was also very interested in the business side of science. So, he went back to New York to get an MBA from Cornell Business School. Upon receiving his MBA, he was accepted into a 3-month rotational training program in sales and marketing at Genentech. His first rotation was in the field doing direct sales to physicians. Charles visited approximately 125 physicians, which gave him a sense of perspective that he never had before about how science affects the lives of individuals. The connection between scientific research, people in the real world, and business became clear to him. He is now in a second rotation at Genentech headquarters in South San Francisco, CA, working in a marketing department. Experience out in the field, combined with his rich background in science, has given Charles great insight into what customers want and need. Charles' advice is to realize that your education can be applied at any level. His experiences as a research scientist have given him a solid foundation for a career in sales and marketing.



How To...

Set Up a Restriction Digest

To set up a restriction digest, the components and quantities need to be determined. First, calculate the necessary volume of DNA. This depends on the concentration of the DNA sample. For example, if 1 µg of DNA is to be digested in a 20 µl reaction volume and the DNA stock is 0.1 µg/µl, then 10 µl of DNA should be used.

Second, calculate the amount of restriction enzyme required. This depends on the concentration of the enzyme, which typically is printed on the enzyme tube label. In a digest, 10 U of enzyme are commonly used per µg of DNA. For example, if the enzyme concentration is 10,000 U/ml, then 1 µl of enzyme contains 10 U and, 1 µl of enzyme should be added to the reaction.

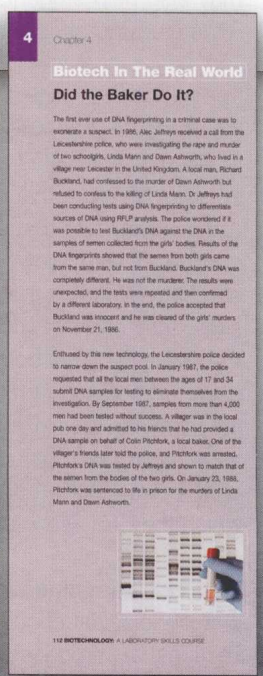
Third, determine the type and volume of restriction digestion buffer. Most restriction buffers are provided at a 10x concentration and are generally packaged with the enzyme. If a digestion using two enzymes is to be performed, look up the manufacturer's recommendation to determine the appropriate buffer for your application.

Finally, calculate the amount of water required to bring the reaction up to the final volume.

To set up the reaction, add the calculated volume of the components to a microcentrifuge tube in the following order: water, buffer, DNA, and, last, enzyme. Mix the components by pipetting up and down, and pulse-spin the tube in a microcentrifuge to collect the contents at the bottom. Incubate the reactions at 37°C in a water bath or dry bath for 30–60 minutes. Place the tube at 4°C until analysis on an agarose gel.

Table 4.1 **Components required for a typical 20 µl restriction digestion reaction.**

Concentration of Stock	Quantity or Concentration	Volume in Digestion Reaction
DNA stock (0.1 µg/µl)	1 µg	10 µl
10x reaction buffer	1x	2.0 µl
Enzyme (10 U/µl)	5 U	0.5 µl
Molecular biology grade water	x µl (to bring up to final volume)	7.5 µl
	Total	20 µl



Biotech In The Real World

Did the Baker Do It?

The first ever use of DNA fingerprinting in a criminal case was to exonerate a suspect. In 1986, Alec Jeffreys received a call from the Leicestershire police, who were investigating the rape and murder of two schoolgirls, Linda Mann and Dawn Ashworth, who lived in a village near Leicester in the United Kingdom. A local man, Richard Buckland, had confessed to the murder of Dawn Ashworth but refused to confess to the killing of Linda Mann. Dr. Jeffreys had been conducting tests using DNA fingerprinting to differentiate sources of DNA using RFLP analysis. The police wondered if it was possible to test Buckland's DNA against the DNA in the samples of semen collected from the girls' bodies. Results of the DNA fingerprints showed that the semen from both girls came from the same man, but not from Buckland. Buckland's DNA was completely different. He was not the murderer. The results were unexpected, and the tests were repeated and then confirmed by a different laboratory. In the end, the police accepted that Buckland was innocent and he was cleared of the girls' murders on November 21, 1986.

Enthused by this new technology, the Leicestershire police decided to narrow down the suspect pool. In January 1987, the police requested that all the local men between the ages of 17 and 34 submit DNA samples for testing to eliminate themselves from the investigation. By September 1987, samples from more than 4,000 men had been tested without success. A villager was in the local pub one day and admitted to his friends that he had provided a DNA sample on behalf of Colin Pitchfork, a local baker. One of the villager's friends later told the police, and Pitchfork was arrested. Pitchfork's DNA was tested by Jeffreys and shown to match that of the semen from the bodies of the two girls. On January 23, 1988, Pitchfork was sentenced to life in prison for the murders of Linda Mann and Dawn Ashworth.

Four types of vignettes show how biotechnology concepts covered in the chapter play a role in our daily lives. Vignette topics include careers, real-life case studies, discussions about bioethics, and spotlights on key skills.

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 with 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. 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).

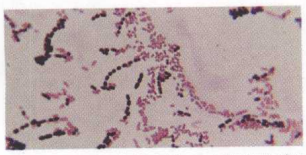


Figure 3.32. **A mixture of *E. coli* and bacteria from yogurt.** The mixture was Gram stained and viewed under an oil immersion lens at 1,000× magnification.

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 clothespins can be used to hold slides while staining and flaming; these clothespins can lower the chance of getting the stain on your fingers.

Safety Reminder: Review the MSDSs of all the stains used in this activity. Before placing any stains on the benchtop, ensure that flaming of slides is complete and that 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 are 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

Refer to Laboratory Skills Assessment Rubric for more details.

- Perform Gram staining of bacteria
- Heat fix bacteria to a slide
- Observe bacteria using a microscope (Activity 3.3)
- Differentiate gram-positive bacteria from gram-negative bacteria
- Identify bacteria from cell shape
- Sketch microscopic details
- Estimate the size of cells using a microscope

Student Workstation Materials

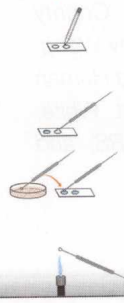
Items	*Quantity*
Microscope and optional accessories, including immersion oil and lens cleaning tissue	1
Stage micrometer (optional)	1
Microscope slide	
Bunsen burner	
Inoculation loop*	
Wooden clothespin (optional)	
Tissue or paper towel	
Wax pencil	
Microbial waste container	
Sterile water	
Beakers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

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

Prelab Focus Questions

1. **What part of the bacterium does the Gram stainin procedure stain?**
2. **Describe the three basic shapes of bacteria and scientific terms used to describe these shapes.**
3. **How do you determine the magnification when u 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.
5. Transfer the loop into the water in the left circle on the microscope slide and swirl the loop to mix the sample with the water.
6. Flame the metal inoculation loop again or obtain a new sterile plastic loop. Transfer a colony

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 an 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.

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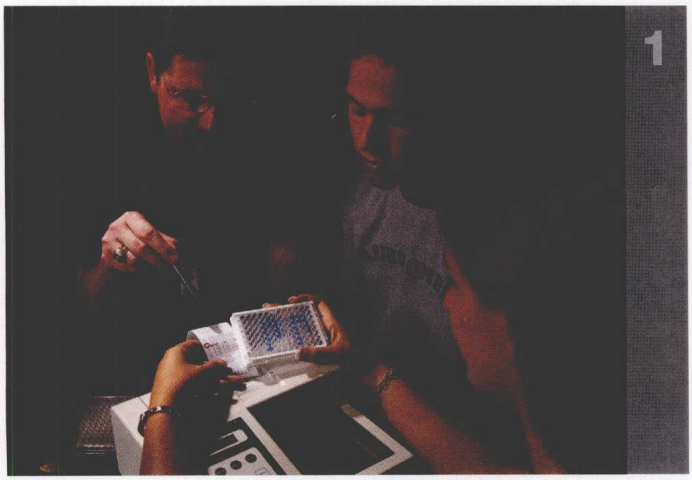
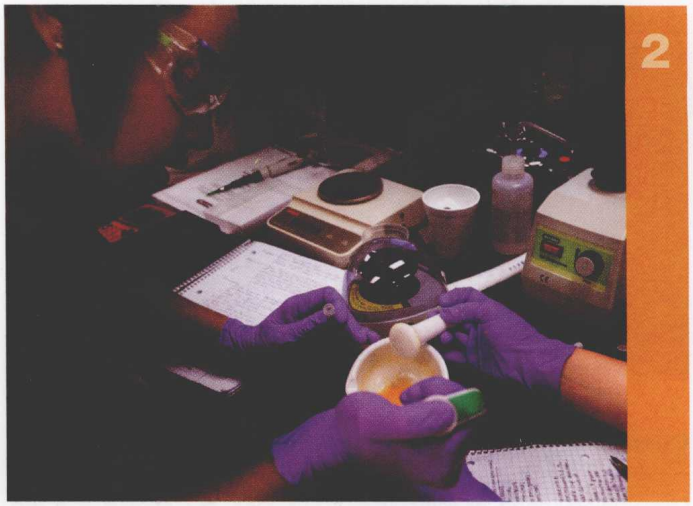
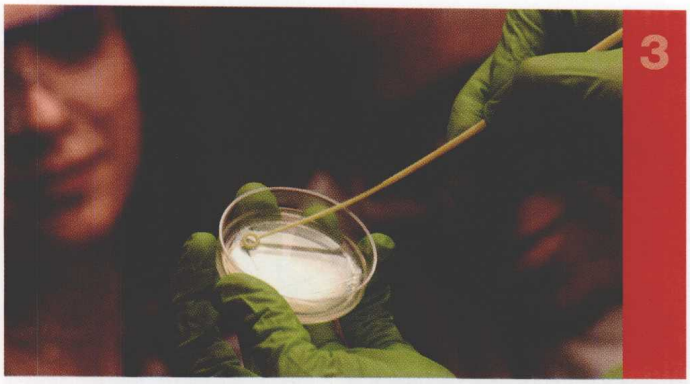
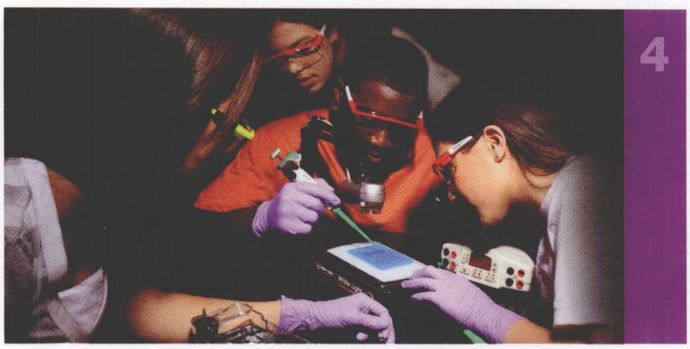
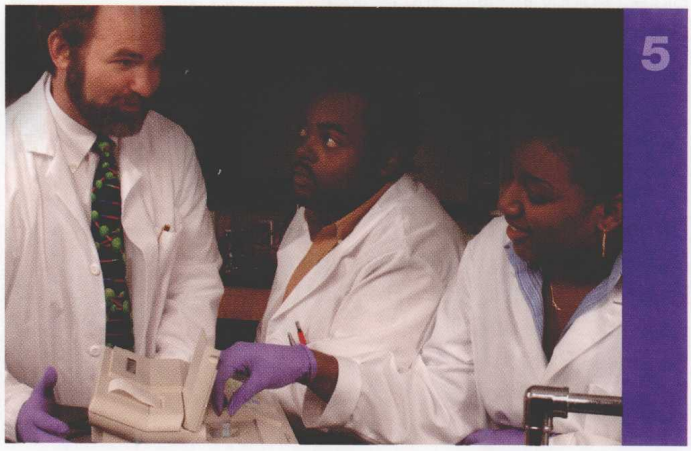
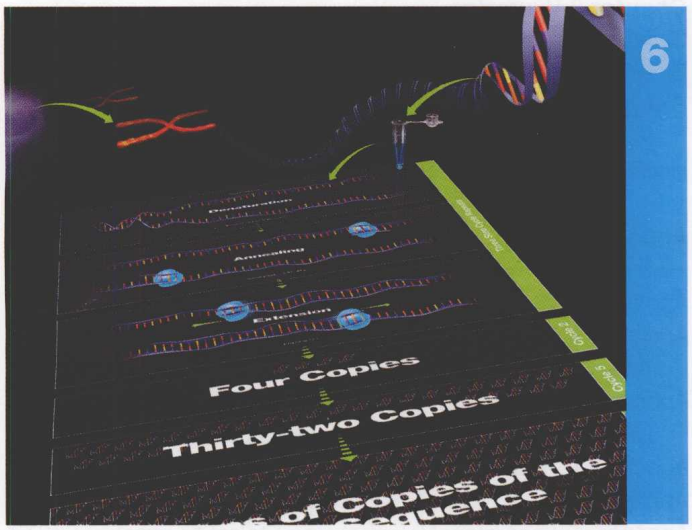


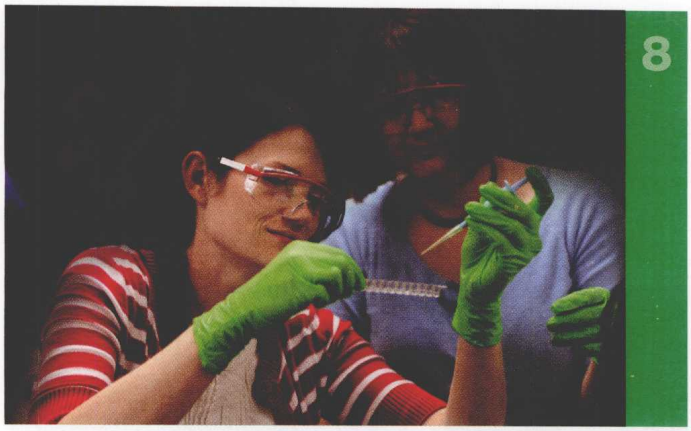


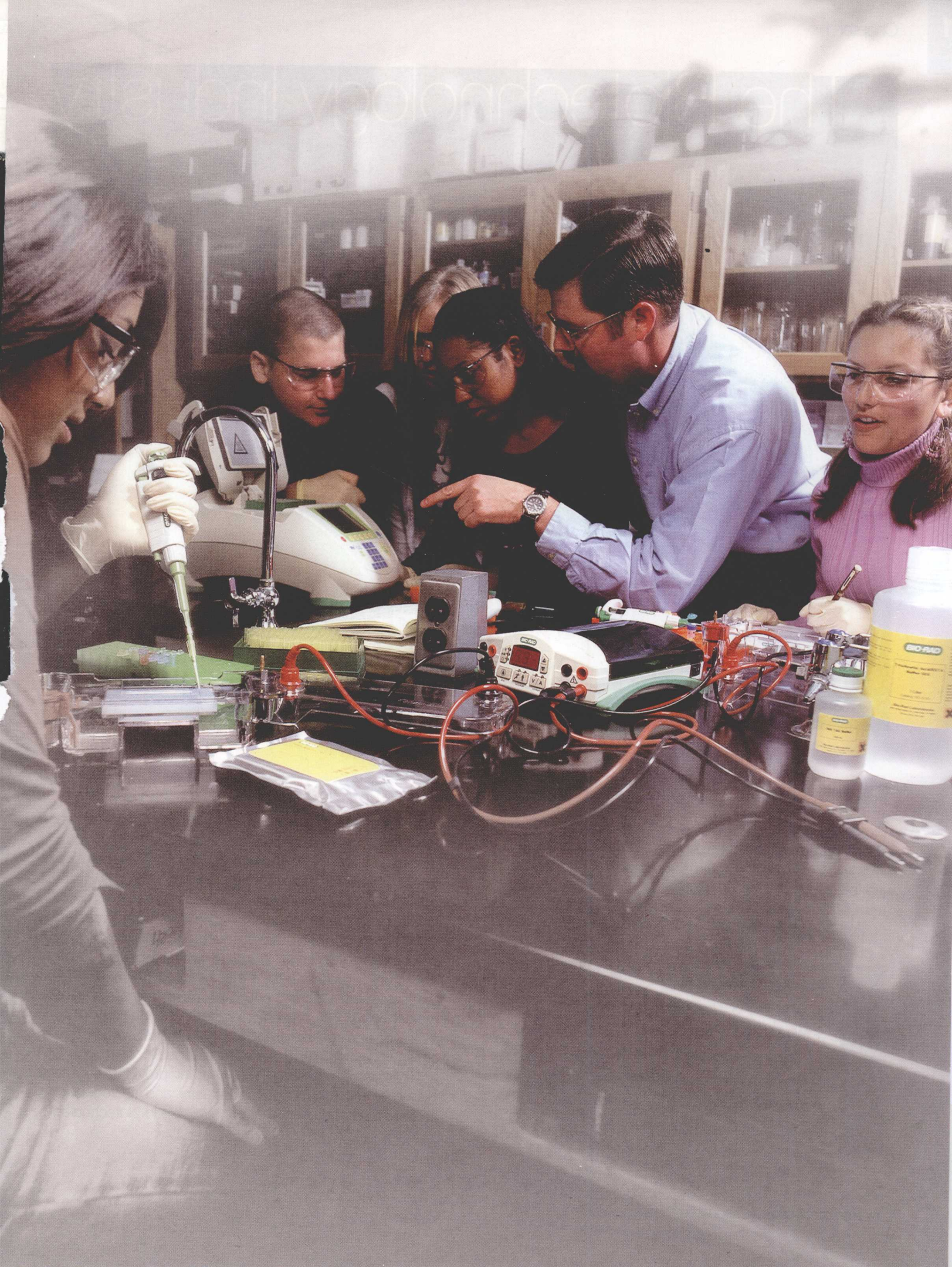




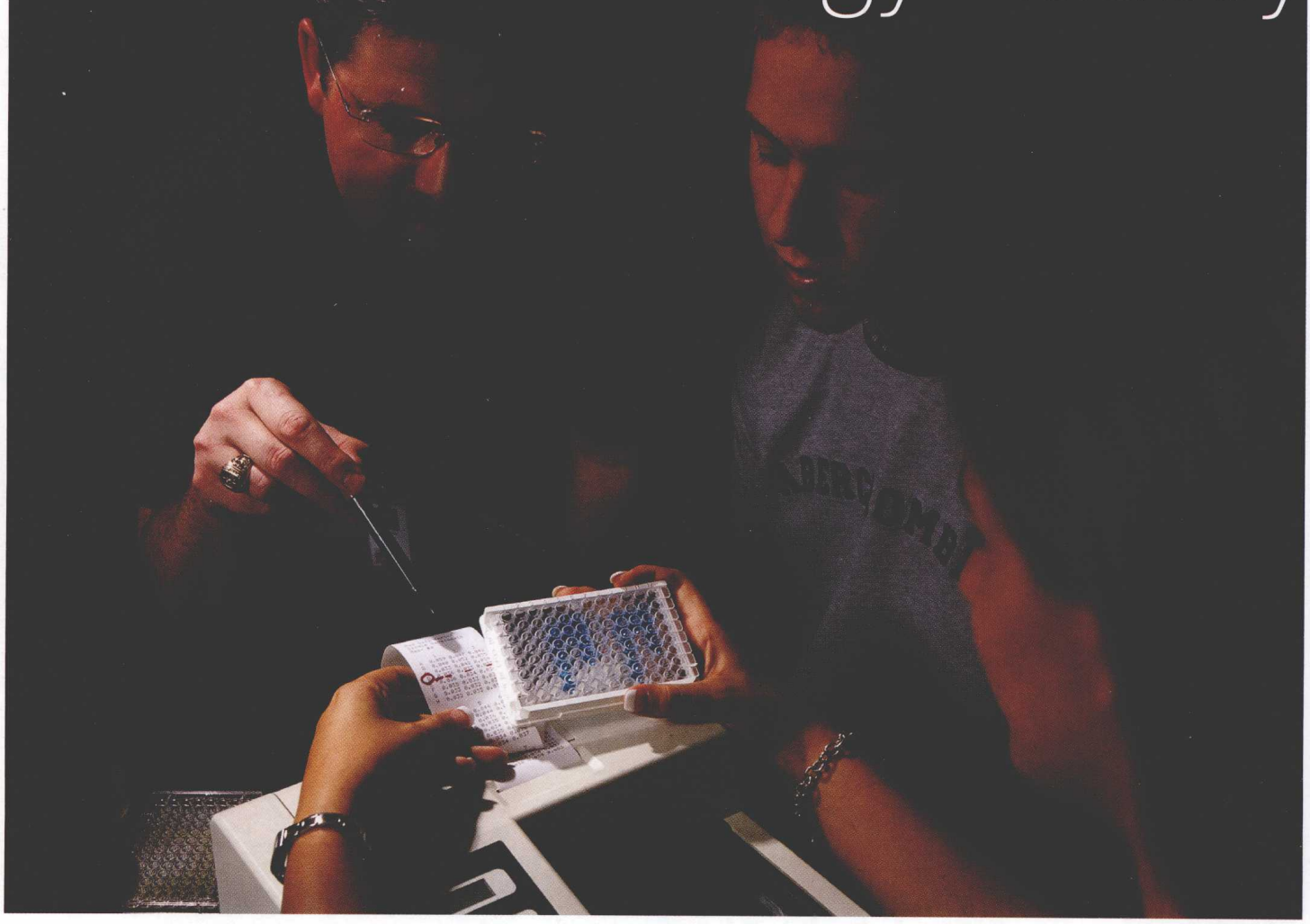







BIO-RAD

The Biotechnology Industry



Summary

Biotechnology is the merging of technology and biology. The products of biotechnology are utilized in most peoples' lives every day. For example, yogurt, bread, and soy sauce are products of biotechnology that have been made for thousands of years. Today, biotechnology products include cutting-edge therapeutic drugs that target cancer as well as enzymes added to laundry detergents that remove fat stains from clothes. The biotechnology industry is in rapid growth after its beginnings in the late 1970s, and the number of career opportunities in the biotechnology sector is increasing. Biotechnology is used in many industries, including biological research, health care, agriculture, and manufacturing. Since the products of biotechnology are used around the world, standard practices such as good laboratory practice (GLP) are required to ensure that products from different countries have the same quality. Biotechnology is powerful; it can change the genetic makeup of organisms including humans, which raises ethical questions and necessitates public oversight and governmental regulation. This chapter provides a general outline of biotechnology, the industry and its regulations, and the type of careers that are available in biotechnology.

Chapter 1: Overview

- What Is Biotechnology?
- Who Uses Biotechnology?
- Biotechnology Industry and Research
- Governmental Regulation of Biotechnology
- Industry Practices
- Careers in Biotechnology

What Is Biotechnology?

Have you ever eaten yogurt, done laundry, worn contact lenses, written on paper, or eaten a puffed corn snack? If so, you have used products of biotechnology. **Biotechnology**, in its broadest sense, is technology based on biology (see Figure 1.1). Biotechnology seems to be a modern phenomenon; however, thousands of years ago humans were applying technology to living things by employing selective breeding to improve yield in plants and utilizing microbes to make yogurt, bread, and wine. A timeline is shown in Figure 1.2 that demonstrates the rapid expansion of biotechnology in the last 150 years.

> **Bi·o·tech·nol·o·gy:** Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Figure 1.1. **Definition of biotechnology from the United Nations Convention on Biological Diversity in 1992.** This definition was expanded in 2003.

Nowadays, biotechnology is considered separate from traditional animal breeding, plant selection, and traditional fermentation. Biotechnology is the use of modern molecular and microbial techniques to make useful products or processes. There is no definitive definition of biotechnology since it is constantly changing; however, a thorough definition of biotechnology was provided by the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (2003), an international treaty on biotechnology. In the treaty, modern biotechnology is defined as the application of techniques "that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection." These techniques include "**in vitro** nucleic acid techniques, such as recombinant DNA or direct introduction of nucleic acid into cells or organelles, or the fusion of cells beyond the taxonomic family."

Biotechnology is not a pure scientific discipline. It draws on knowledge and techniques from many of the biological sciences, such as genetics, molecular biology, biochemistry, cell biology, and microbiology (see Figure 1.3). Biotechnology also draws on many nonbiological fields of study, such as engineering, chemistry, physics, and information technology. Conversely, many of these disciplines also draw on the methods that biotechnology has developed (see Figure 1.4). Biotechnology is distinguished from other biological disciplines by its purpose to develop products and processes. Because commercial products are created by the biotechnology industry, biotechnology makes a direct contribution to the world's economy.

The Biotechnology Toolkit

Biotechnology is fundamentally a set of tools comprised of a series of techniques that can help solve a multitude of problems. These tools can be biological, chemical, instrumentation, or software. Biotechnology companies such as Bio-Rad Laboratories are in the business of making new and better tools and techniques for researchers and businesses.

Bioethics

Biotechnology: Good or Bad?

Biotechnology is powerful. It can change the genetic makeup of organisms including humans. Because of this power, biotechnology is controversial and anti-biotechnology sentiment is common. Anti-biotechnology activists argue that biotechnology is against nature and that biotechnologists may misuse their power. Opponents of biotechnology also express concern that biotechnology companies consider their profits before taking into account the impact of their technology on the planet and on human life. Some concerns are unfounded and based on fear rather than scientific data, for example that genetically modified food will genetically modify the person eating it. However, biotechnology has real ethical implications that should be considered in the context of real scientific data.

Biotechnologists themselves appreciate the power of the technology. When the implications of DNA technology first became evident, the National Academy of the Sciences organized a conference in Asilomar, CA, in 1975. The participants drew up principles and guidelines for conducting recombinant DNA experiments to minimize biohazards that are generated during the experiments. The conference organizers also brought the implications of the technology into the public domain to encourage discussion. Today, the ethical implications of new technologies are still central to all scientific discussion.

Where would you draw the line? Would you accept cancer therapy with a drug made from a genetically engineered virus? Would you drink milk from a cow treated with a recombinant growth hormone? Would you eat genetically modified fish? Would you clone your pet? Would you genetically modify a pre-implantation embryo to fix a genetic disease? Would you clone yourself? Consider these questions as you learn more about the power of biotechnology. Use the knowledge you gain in this course to make informed decisions in your personal and professional life.

Many tools use whole cells or molecules, such as DNA, RNA, and proteins that are derived from nature. Scientists modify these cells and molecules to perform new tasks. Chemistry is also important in biotechnology since many tools rely on chemical interactions. Laboratory instruments are used to perform and analyze biotechnological procedures and products. Advances in biotechnological techniques are frequently made by finding new ways to use or improve existing tools. The activities in this book will introduce you to many of the tools and techniques used in biotechnology.

Biological tools include enzymes that cut and reattach DNA. These enzymes allow scientists to transfer DNA from one organism to another so that the recipient organism can perform a new and useful task. For example, the human insulin gene was cut out of the human genome and inserted into bacteria to give the bacteria the ability to make human insulin. The insulin was subsequently purified and is now used as a therapy for diabetes. The process of moving a gene from one organism and expressing it in another is called **genetic engineering**. Genetically engineered bacteria and eukaryotic cells are biological tools that are frequently used as factories to produce novel proteins (**recombinant proteins**).

Chemical tools include **chromatography** resins that bind proteins based on particular properties of the proteins, allowing them to be purified from other cellular components. For example, chromatography resins are used by pharmaceutical companies to purify biological drugs produced in bacteria.

Biotechnology relies on many instruments that differ based on the application. Some instruments measure, while other instruments perform a function. **Spectrophotometers** measure the amount of light absorbed by a solution and are used to quantitate bacteria, proteins, and DNA. **Thermal cyclers** rapidly heat and cool tubes of DNA and enzymes, thereby enabling rapid DNA replication by a technique called the **polymerase chain reaction** (PCR). Among many other applications, PCR is used in forensic laboratories to fingerprint DNA.

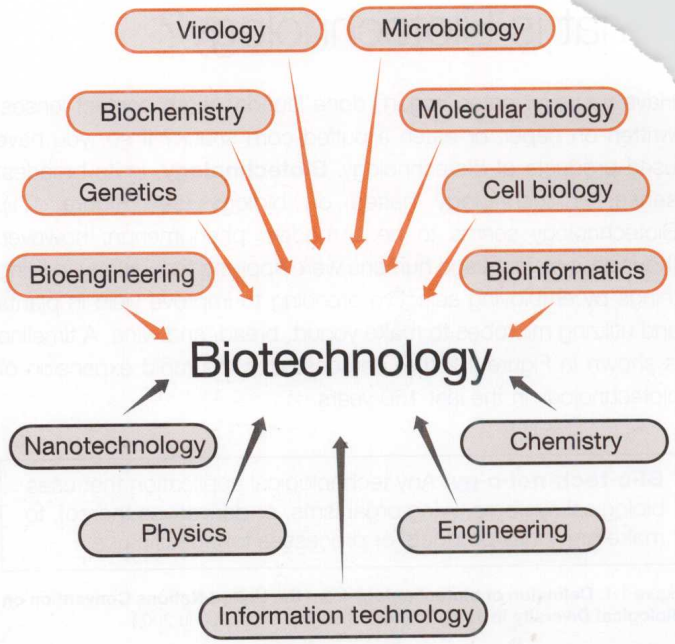


Figure 1.3. **Scientific disciplines that contribute to biotechnology**. Orange boxes represent biological sciences and gray boxes represent other scientific disciplines.

Biotechnological tools and techniques are constantly being improved and applied in new and exciting ways to help solve the problems of mankind.

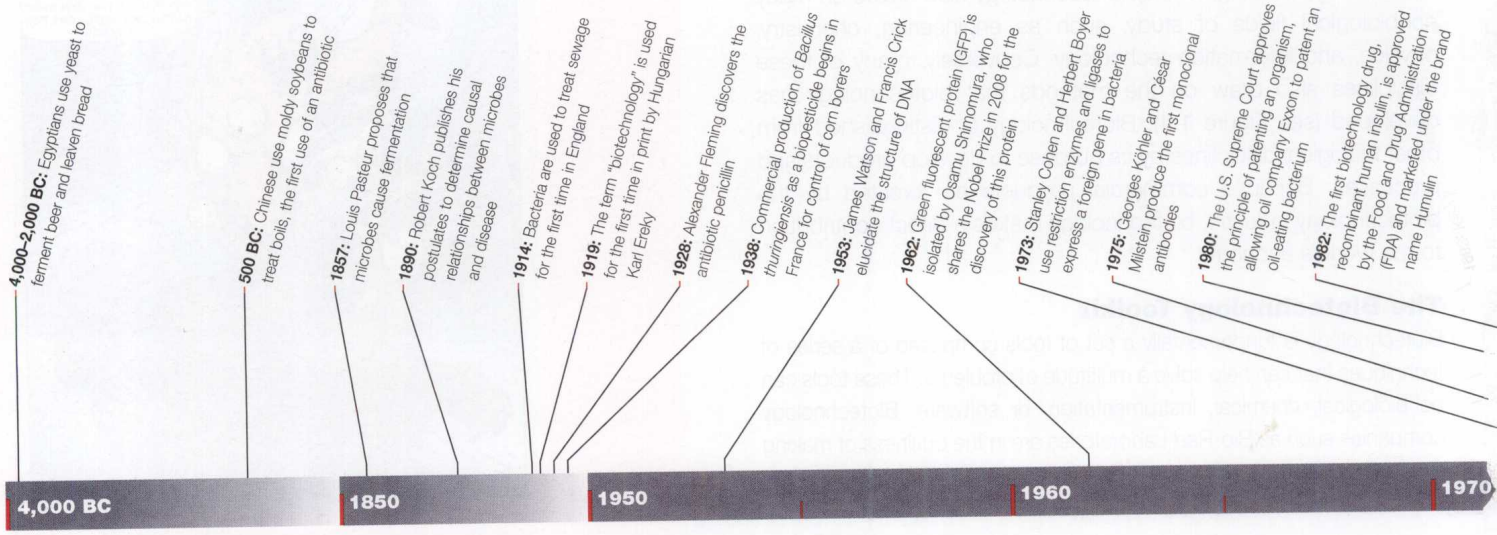


Figure 1.2. **Timeline of biotechnology**.