AMGEN® Biotech Experience

Scientific Discovery for the Classroom

FOUNDATIONS OF BIOTECH

ABRIDGED GENETIC ENGINEERING SEQUENCE

Teacher Guide

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AMGEN® Foundation

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PROGRAM HISTORY

THE PIONEERS OF THE AMGEN BIOTECH EXPERIENCE

"Pioneers lay the roads for those who follow to walk on."

—Unknown

Though the Amgen Biotech Experience now reaches nearly 100,000 students and 1,500 teachers each year, the program had humble beginnings. It all started with a group of scientists and teachers who had a passion for sharing their knowledge with students.

In 1989, molecular biologist Bruce Wallace, Scientific Executive Director Steve Elliot (now retired), and others at Amgen believed that the company could be instrumental in providing professional development to area high school teachers, toward a goal of improving science education for students. They put out a call for biology teachers interested in a summer intern program.

Intrigued, Hugh Nelson, a high school teacher in Thousand Oaks, Calif., responded to their invitation. Nelson set about learning the procedures Amgen uses to develop biologics, and worked with an Amgen scientist to fine-tune a series of labs for high school students. Amgen agreed to provide equipment and chemicals to teach the lab procedures in area high schools.

Two years later, Amgen launched the official school program in Ventura County, Calif., schools. Within two years of the launch, 1,300 students from 12 local schools participated in the program.

"The labs put students in touch with the reality of modern science," says Nelson. "It takes money to do experiments, and Amgen provided the funding for this important, transformative program. I'm no less in awe of the program [now] than I was in 1989."

In 1999, Amgen enlisted Marty Ikkanda, professor of biological sciences at Pierce College in Woodland Hills, Calif., to revise the program's curriculum to resemble

his college classes. The revised program was rolled out to 20 schools the next school year, and the number reached 30 by the school year's end.

"Teachers tell us they don't have attendance problems when they're doing the Amgen biotechnology labs," says Ikkanda, who retired from the program in 2013. "It's a fantastic way to interest students in science."

In 2005, with interest building from biology teachers in other communities, the Amgen Foundation, the main philanthropic arm of Amgen, partnered with Professor Ikkanda to expand the program. Over several years, the program expanded to new Amgen communities in the United States and Europe.

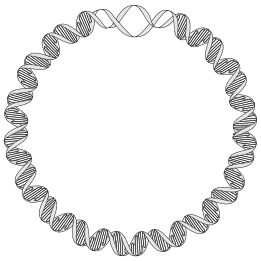
In 2013, the Amgen Foundation joined forces with Education Development Center, a global nonprofit organization with deep experience and expertise in science education, to establish a Program Office to support and strengthen the program. The program has continued to expand into new Amgen communities internationally.

A collaboration that began nearly 30 years ago inspired the ongoing commitment of scientists and teachers to share their knowledge of and passion for science. The Amgen Foundation is proud to continue its support of a program that is stronger than ever—and poised to bring real-world biotechnology to a new generation of teachers and students. "That pioneering spirit distinguishes Amgen," says Eduardo Cetlin, president of the Amgen Foundation. "We're forever grateful to those early collaborators for the roots of this powerful program."

Visit the ABE website at www.amgenbiotechexperience.com.

TABLE OF CONTENTS

REPRODUCIBLE MASTERS (RMS)



CURRICULUM OVERVIEW

INTRODUCTION

Integrating ABE into your classroom is a unique and important opportunity for you and your students. To support you in developing students' scientific content knowledge and practices, as well as providing a real-world application, the material in this section covers the *what, why, and how* of the curriculum: the science content addressed, the teaching and learning principles and practices employed, and practical considerations for implementing the course. Reading this section *before* you begin will enhance your teaching and the benefits that students derive from it.

The ABE labs parallel some of the important steps the pharmaceutical biotechnology industry uses to manufacture drugs (therapeutic proteins) to treat a variety of diseases. Biotechnology provides the tools and techniques for modern pharmaceutical research and drug development, and it is critical that future citizens are knowledgeable about this field. Common biotechnology techniques are used to develop a wide range of products, from life-saving insulin to an enzyme that increases the vitamin content of rice to a human vaccine expressed in a plant. The labs focus on these techniques so that your students will better understand the tools of biotechnology and can begin to consider the potential impact of this industry on our future. In addition, by engaging in this program, students may be more motivated to understand the underlying science concepts and perhaps even pursue careers in science.

Laboratory experiences such as those provided by this program are extremely important in science education, as they offer opportunities to make science "real" and relevant to students and allow them to use the professional tools and techniques of biotechnology to investigate scientific questions. Effective laboratory experiences can do the following:

- Enhance students' understanding of fundamental science content and concepts
- Help students develop scientific skills and reasoning practices
- Increase students' understanding of the complexity and ambiguity of scientific research

- Allow students to develop important academic, interpersonal, and intrapersonal skills that are necessary for their future success, including problem-solving, critical thinking, and teamwork

CONTENT OF THE ABE CURRICULUM

In the Abridged Genetic Engineering Sequence, students work with a recombinant DNA molecule that is a chimera of prokaryotic and eukaryotic genetic elements. The prokaryotic component consists of an engineered bacterial plasmid and its control elements—origin of replication, ampicillin-resistant gene, and the arabinose operon—while the eukaryotic element is a gene from the sea anemone, *Discosoma* sp. The gene encoding this protein is referred to as the red fluorescent protein (*rfp*) gene. The *rfp* gene encodes the mutant fluorescent protein, mFP, a molecule that is used extensively in research. (Note that the mFP protein is called RFP in the Student Guide.)

Once students have studied the recombinant DNA plasmid, they use it to transform *Escherichia coli* (*E. coli*). The transformed cells are spread on the surface of an agar plate containing ampicillin and arabinose, a five-carbon monosaccharide required for *rfp* gene expression. Bacterial colonies expressing the *rfp* gene will appear red (or bright pink) in color. If students are carrying out the optional Laboratory 6, cells from one of these red colonies are transferred to a liquid medium culture, and the cells are harvested and lysed (broken open) to release the red fluorescent protein (RFP) into the solution. This solution is then fractionated on a chromatography column, and RFP is purified from other proteins in the cell lysate based on its hydrophobicity.

The process for making a human therapeutic protein is very similar, though colonies of recombinant bacteria cannot be identified by their expression of RFP. When making a human therapeutic protein, another step must occur: Colonies that grew on the plate containing ampicillin must then be cultured on a plate containing another antibiotic, tetracycline. The gene for tetracycline is interrupted when the human gene is introduced, and bacteria from recombinant colonies will not survive on the plate that contains tetracycline. Cells from colonies that could not survive on tetracycline plates are then cultured and lysed to release the human therapeutic protein, which is then purified.

By completing this sequence, your students will have a clearer understanding of how to use recombinant DNA techniques to introduce new genes into an organism and have that organism produce new proteins. Using a gene from a sea anemone to express the red fluorescent protein models how the same process can be used with a human gene to produce proteins for a human therapeutic protein, such as insulin or human growth hormone.

The ABE program includes multiple lab sequences to suit a variety of classroom situations. In all four sequences, students read the introductory reading and

complete the first set of labs. Each of the ABE lab sequences is described in **Table OV.1** below.

Table OV.1: Possible ABE Lab Sequences and Content Covered in Each

Sequence	Content	# of Sessions
Abridged Genetic Engineering Sequence (16–18 sessions)	**Introduction:** Students read about the biotechnology and biopharmaceutical industries.	2
	Lab 1: Students learn how to use two basic biotechnology lab tools: micropipettes and gel electrophoresis	2–3
	Lab 2A: Students learn how plasmids are made. In the lab, students use restriction enzymes to create DNA fragments that contain the *rfp* gene.	3–4
	Lab 4A: Students verify that they have the correct recombinant plasmid.	3
	Lab 5A: Students transform the bacteria using the recombinant plasmid, then grow the transformed bacteria.	3
	Lab 6 (optional): Students extract and purify the protein made within the bacteria by the *rfp* gene.	3
Complete Genetic Engineering Sequence (18–20 sessions)	**Introduction:** Students read about the biotechnology and biopharmaceutical industries.	2
	Lab 1: Students learn how to use two basic biotechnology lab tools: micropipettes and gel electrophoresis	2–3
	Lab 2: Students learn how plasmids are made. In the lab, they make a plasmid that contains the *rfp* gene.	3–4
	Lab 3: Students ligate their plasmid.	2
	Lab 4: Students verify that they have the correct recombinant plasmid.	3
	Lab 5: Students transform the bacteria using the recombinant plasmid, then grow the transformed bacteria.	3
	Lab 6 (optional): Students extract and purify the protein made within the bacteria by the *rfp* gene.	3
Focus on Bacteria Sequence (12–14 sessions)	**Introduction:** Students read about the biotechnology and biopharmaceutical industries.	2
	Lab 1: Students learn how to use two basic biotechnology lab tools: micropipettes and gel electrophoresis	2–3
	Lab 5B: Students learn how plasmids are made. In the laboratory, they add a plasmid that contains the *rfp* gene to bacteria, thereby creating a genetically-engineered organism. They grow the bacteria.	5–6
	Lab 6 (optional): Students extract and purify the protein made within the bacteria by the *rfp* gene.	3
Introduction to Biotechnology Sequence (4–5 sessions)	**Introduction:** Students read about the biotechnology and biopharmaceutical industries.	2
	Lab 1: Students learn how to use two basic biotechnology lab tools: micropipettes and gel electrophoresis.	2–3

TIMING SUGGESTIONS FOR TEACHING ABE

Given the short amount of time that you're allowed to keep the kit, it may be challenging to complete the full sequence of ABE labs during the loan period. With the Abridged Genetic Engineering Sequence, you should complete the Program Introduction before you receive the kit.

OVERVIEW OF CONTENT

The Curriculum Overview describes the science content of the curriculum that your students should ideally be engaged in while completing the ABE program. Given that you may have the kit at a time when you are teaching other content, from ecology to evolution—or that you may even use these labs with courses other than biology—it is especially important to provide context for and connections to the science curriculum while students are engaged in the lab experiences.

THE BIG IDEA

Proteins encoded by DNA are responsible for traits:

THE BIG QUESTION

What is the relationship between genes, proteins, and the traits of an organism?

SUB-QUESTIONS

1. How does the expression of a gene result in the traits of an organism?
2. What is the relationship between proteins and traits?
3. How is information encoded in DNA?
4. How is this information decoded?
5. What is the product of the decoded message?
6. How does this product result in traits?
7. What are the consequences to an organism if the product is altered or not made?
8. How can organisms be engineered to make different protein products and have new traits?

GOALS FOR UNDERSTANDING

- Students understand that all the information required by organisms to maintain life is encoded in the arrangement of nucleotides in their DNA.
- Students understand that the coding and decoding of DNA is the same among all organisms, which makes possible the expression of a human gene by bacteria.
- Students understand how the processes of transcription and translation facilitate the transfer of information from DNA to proteins.
- Students recognize that the traits of organisms are determined by the expression of specific genes in their DNA.
- Students understand how gene expression is regulated.
- Students understand how protein functions are responsible for the traits of an organism.
- Students recognize that a change in the DNA sequence can alter the function of a protein and can change the traits of an organism.
- Students know how bacteria can be genetically modified to make new products.
- Students understand the reciprocal relationship between basic science research and technology development: They understand that the discoveries of plasmids, restriction enzymes, and ligases during basic research have generated the tools and techniques of biotechnology; in turn, the tools and techniques of biotechnology are enabling scientists to reach deeper understandings about genes and the function of their products in a cell.
- Students understand the purpose of using controls in scientific investigations.

LEARNING OUTCOMES

After completing the program, students should be able to do the following:

- Describe the relationship between DNA, genes, proteins, and traits
- Explain how information is transferred from DNA to proteins by transcription and translation
- Explain how the expression of genes in a cell determines the traits of an organism
- Describe how the characteristics of organisms (traits) are the result of protein activity
- Describe how a loss or gain of protein function can alter the traits of an organism
- Discuss how organisms can be engineered to have new characteristics
- Describe the role of biological tools, such as plasmids, restriction enzymes, and DNA ligase, in the genetic engineering process
- Provide examples of how genetic engineering can be used to solve medical problems
- Model the process of producing a recombinant plasmid

CONNECTIONS TO BIOLOGY CURRICULUM

Here are suggestions as to where the ABE labs can be integrated appropriately into the conceptual flow of classroom science instruction.

Chapter	Curriculum Connections	Notes
1	Laboratory skills	• Use of micropipettes • Use of gel electrophoresis
2A	• DNA structure • Genes and DNA • Function of enzymes	• Basic structure of DNA • Principles and uses of genetic engineering (GE) • Relationship between genes and DNA • How enzymes work • Restriction enzymes
4A	Universality of DNA	Visualizing DNA
5A	• Universality of gene expression • Transcription • Translation • Protein activity • Bacteria cell structure • Variables and controls	• Biology big idea: DNA → Protein → Trait • Process of gene expression • All organisms express genes the same way • Colonies vs. individual cells
6	• Bacterial growth • Protein structure and function • Biomolecules • Mutation • Evolution	• Exponential growth, lag, death phase • Protein folding • Biochemistry • *rfp* gene mutation • Evolutionary reason for fluorescence

CONNECTIONS TO ACADEMIC STANDARDS

LINKS TO CORE BIOLOGY CONTENT

ABE meets many of the Next Generation Science Standards (NGSS) and the Common Core State Standards (CCSS). The biology explored by students in the program is core to the learning of science and technology. Students gain content knowledge, proficiency with scientific practices, and understanding of several disciplinary core ideas. Students also explore scientific reading and writing that meets the CCSS English Language Arts standards.

The following symbols denote when a concept is introduced, developed more fully, or elaborated on:

* = Introduced

** = Developed

*** = Elaborated on

NEXT GENERATION SCIENCE STANDARDS

Performance Expectations	Chapter					
	Intro	1	2A	4A	5A	6
HS-LS1. From Molecules to Organisms: Structures and Processes						
HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.	*	*	**		***	
HS-LS1-6. Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules.			*			***
HS-LS3. Heredity: Inheritance and Variation of Traits						
HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.			*		***	
HS-LS3-2. Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.						
HS-LS4 Biological Evolution: Unity and Diversity						
HS-LS4-4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations.			**		**	
HS-LS4-5. Evaluate the evidence supporting claims that changes in environmental conditions may result in (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.			**	*	*	

NEXT GENERATION SCIENCE STANDARDS

Scientific Practices	Chapter					
	Intro	1	2A	4A	5A	6
Asking Questions and Defining Problems						
Ask questions that arise from examining models or a theory to clarify relationships.		*	**	**	**	***
Developing and Using Models						
Use models (including mathematical and computational) to generate data to support explanations and predict phenomena, analyze systems, and solve problems.			*		*	
Planning and Carrying Out Investigations						
Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design; decide on types, how much, and the accuracy of data needed to produce reliable measurements, consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly.			*		**	
Constructing Explanations and Designing Solutions						
Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws describing the natural world operate today as they did in the past and will continue to do so in the future.			*	**	**	***
Engaging in Argument from Evidence						
Make and defend a claim based on evidence about the natural world that reflects both scientific knowledge and student-generated evidence.			*	**	**	***

NEXT GENERATION SCIENCE STANDARDS

Crosscutting Concepts	Chapter					
	Intro	1	2A	4A	5A	6
Patterns						
Observed patterns of forms and events guide organization and classification, and they prompt questions about relationships and the factors that influence them.	*					
Cause and effect: Mechanism and explanation						
Events have causes, sometimes simple, sometimes multifaceted. A major activity of science is investigating and explaining causal relationships and the mechanisms by which they are mediated. Such mechanisms can then be tested across given contexts and used to predict and explain events in new contexts.			*		**	
Scale, proportion, and quantity						
In considering phenomena, it is critical to recognize what is relevant at different measures of size, time, and energy and to recognize how changes in scale, proportion, or quantity affect a system's structure or performance.			*	**		
Systems and system models						
Defining the system under study—specifying its boundaries and making explicit a model of that system—provides tools for understanding and testing ideas that are applicable throughout science and engineering.			*	*		
Structure and function						
The way in which an object or living thing is shaped and its substructure determine many of its properties and functions.	*	*	***	***	**	***
Stability and change						
For natural and built systems alike, conditions of stability and determinants of rates of change or evolution of a system are critical elements of study.						*

NEXT GENERATION SCIENCE STANDARDS

Disciplinary Core Ideas	Chapter					
	Intro	1	2A	4A	5A	6
LS1.A: Structure and Function						
Systems of specialized cells within organisms help them perform the essential functions of life.	*	*				
All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.	*	*	**	**	***	***
LS1.C: Organization for Matter and Energy Flow in Organisms						
The sugar molecules thus formed contain carbon, hydrogen, and oxygen. Their hydrocarbon backbones are used to make amino acids and other carbon-based molecules that can be assembled into larger molecules (such as proteins or DNA), which can then be used, for example, to form new cells.		*	**			***
LS3.A: Inheritance of Traits						
Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no known function (thus far).	*	*	**	**	***	
LS3.B: Variation of Traits						
In sexual reproduction, chromosomes can sometimes swap sections during the process of meiosis (cell division), thereby creating new genetic combinations and thus more genetic variation. Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. Environmental factors can also cause mutations in genes, and viable mutations are inherited.					*	
Environmental factors also affect expression of traits and hence affect the probability of occurrences of traits in a population. Thus, the variation and distribution of traits observed depends on both genetic and environmental factors.			*			

NEXT GENERATION SCIENCE STANDARDS

Disciplinary Core Ideas	Chapter					
	Intro	1	2A	4A	5A	6
LS4.B: Natural Selection						
The traits that positively affect survival are more likely to be reproduced, and thus are more common in the population.			*		*	
LS4.C: Adaptation						
Natural selection leads to adaptation, that is, to a population dominated by organisms that are anatomically, behaviorally, and physiologically well suited to survive and reproduce in a specific environment. That is, the differential survival and reproduction of organisms in a population that have an advantageous heritable trait leads to an increase in the proportion of individuals in future generations that have the trait and to a decrease in the proportion of individuals that do not.			* *		* *	

COMMON CORE STATE STANDARDS

English Language Arts/Literacy	Chapter					
	Intro	1	2A	4A	5A	6
RST.11-12.9: Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.		*	*	**	**	***
WHST.9-12.1: Write arguments focused on discipline-specific content.			*	**	**	***
WHST.9-12.9: Draw evidence from informational texts to support analysis, reflection, and research.		*	**	**	**	***

LINKS TO 21ST CENTURY CORE COMPETENCIES[1]

The ABE program allows many opportunities for students to develop 21st Century Core Competencies in the cognitive and interpersonal domains.

Cognitive Domain	Chapter					
	Intro	1	2A	4A	5A	6
Cognitive Processes and Strategies						
Critical thinking				**	**	***
Problem solving			*	**	**	***
Analysis			*	**	**	***
Reasoning/argumentation		*	**	**	**	***
Interpretation		*	**	**	**	***
Knowledge						
Oral and written communication		*	**	**	**	***
Active listening		*	**	**	**	***

Interpersonal Domain	Chapter					
	Intro	1	2A	4A	5A	6
Teamwork and Collaboration						
Communication		*	**	**	**	***
Collaboration		*	**	**	**	***
Teamwork		*	**	**	**	***
Interpersonal skills		*	**	**	**	***

[1] From National Research Council. (2012). *Education for life and work: Developing transferable knowledge and skills in the 21st century*. Washington, DC: The National Academies Press.

ASSUMPTIONS OF PRIOR KNOWLEDGE AND SKILLS

The ABE labs can be used for any biology class with minimal adaptation. However, students at the beginning of an introductory biology course may have less conceptual understanding of some of the science concepts connected to these labs and will therefore require more support in making those connections. We encourage you to teach these concepts as students need them, rather than teach them all before beginning the program. Prior knowledge assumed for each chapter is listed at the beginning of each chapter in this guide.

Students with little experience in conducting science labs may need additional time and instruction in order to have a safe and effective lab experience.

ASSESSING STUDENT LEARNING

You can assess students' understanding throughout the program as they participate in class discussions and answer the lab questions. The *STOP AND THINK* questions in the laboratory *Methods* section are designed to have students consider their thinking at that point and time; these questions also give you a snapshot of their understanding as they go through the investigation.

At the end of the ABE program, you can pose final assessment questions such as the following (possible answers follow the questions in italics):

1. Explain the purpose, products, tools, and process of genetic engineering. *The purpose of genetic engineering is to address the need to treat diseases that result from the lack of a functional protein. The products associated with genetic engineering are functional proteins. The tools associated with genetic engineering are plasmids, restriction enzymes, and bacteria. The genetic engineering process is the transfer of a human gene into a plasmid, inserting the plasmid into bacteria, cloning the plasmid, and purifying the protein manufactured by the bacteria.*

2. Review **Figure 2A.4** on page 39 of the Student Guide. Where in this process would you use pipetting and gel electrophoresis? *Pipetting is used in every step, and gel electrophoresis is used to confirm that the restriction enzyme digest worked and that the correct recombinant plasmid was made.*

3. How does the cell use the instructions in DNA to make proteins? *The transfer of information from DNA into protein occurs in two steps: transcription and translation.* Transcription *is the copying of the DNA gene onto messenger RNA (mRNA).* Translation *is the synthesis of the protein using the mRNA.*

4. Why is it important for scientists to have both in-depth knowledge of biology and laboratory skills? *To produce a product from a gene, scientists need scientific knowledge of genetic disease, DNA, and cell processes in order to*

design the laboratory procedures, and they need laboratory skills to carry out those procedures.

5. In this program you read about how scientists modify a bacterial plasmid by adding a human gene in order to produce human therapeutic proteins. How does this process help in addressing genetic diseases? *People who lack the human proteins can use the proteins made by bacteria to treat the symptoms of their disease.*

6. Before human insulin was made by genetic engineering, people used insulin extracted from livestock that was similar to (but not exactly the same as) human insulin. What might be the benefits of genetically engineered insulin over insulin extracted from livestock? *Genetically engineered insulin has the following advantages compared to insulin extracted from livestock: (1) It is easier to obtain high quantities, (2) it is exactly the same as human insulin, so it less likely to cause an adverse reaction, and (3) it addresses ethical concerns about using animals.*

7. What are some possible risks that might be associated with using bacterial cells to produce human proteins? *The bacteria could be released into the environment. One possible result of this release is that bacteria that have antibiotic resistance might pass on the resistance to other bacteria, including harmful bacteria. Another risk is that the human gene product made by the bacteria might be slightly different from the protein made in human cells, thus producing risky side effects, or the product might undergo an undetected change and no longer be effective or safe for human use.*

LABORATORY SAFETY

It is critical to teach students proper lab safety during lab experiences. The lab protocols used in the ABE program are designated as Lab Safety Level 1, requiring minimal precautions. However, since students are working with real-world lab equipment, these protocols offer an excellent opportunity to teach students good practices in terms of lab safety. Of particular note, in Chapters 5 and 6 students work with *E. coli* cells. While the strain of cells used in ABE is quite benign, it is important that students follow good practices and take precautions while completing the labs.

All students should know the following laboratory basic safety precautions:

- Tie back long hair.
- Do not eat or drink in the lab, or store food or drinks in the lab.
- Keep lab benches and tables clear of everything except lab materials, notebooks, and implements.
- Handle materials and equipment with care, and never use chemicals and dyes without proper supervision.

- Be sure that all cultures, chemicals, disinfectants, and media are clearly labeled with names and dates. If they are hazardous, include proper hazard information.
- Wear lab goggles at all times.
- Know the location of the nearest eyewash station and sink.
- Cover any cuts on hands with a bandage. Gloves may be worn as extra protection.

Carry out the following safety procedures when working with microorganisms:

- Treat all microorganisms as if they were pathogenic.
- Wear disposable gloves when handling bacteria in the transformation lab (Laboratory 5/5A/5B).
- When handling microfuge tubes, pipette tips, cell spreaders, and Petri plates, avoid spills and any unnecessary contact. Inform the teacher if a spill occurs.
- Wash hands with a disinfectant soap before and after working with microorganisms. Non-disinfectant soap will remove surface bacteria and can be used if disinfectant soap is not available. Gloves may be worn as extra protection.
- **Dealing with laboratory waste:**
  - Place all equipment that comes into contact with bacteria in a well-labeled biohazard bag.
  - Do not put liquid waste in the biohazard bag. Pour liquid waste into a specially designated container.
  - Return the biohazard bag with the kit to be autoclaved and reused.
  - Sterilize the liquid waste by adding 10% bleach solution and then pouring it down the drain.
- **Cleaning up:**
  - Clean up spills immediately, using caution. Soak the spill with a 10% bleach solution and cover with paper towels. After allowing the spill to soak in the bleach for two minutes, carefully clean up and place the materials in the biohazard bag. Wash the area again with disinfectant.
  - Use a 10% bleach solution to disinfect all benches and work areas before and after working with microorganisms.
  - If there are glass fragments, use a specially designated brush and dustpan to sweep them up. Place the fragments in a 10% bleach solution, then drain and dispose of them, according to local regulations.
  - Wear chemical-resistant goggles and be cautious when handling the bleach solution.
  - Know the location of the nearest eyewash station and sink.

  NOTE: Do not let students handle the bleach solution.

MATERIALS PREPARATION

Before you begin, you should become familiar with the lab procedures in each chapter, the preparation required, and the materials you'll need. Materials preparation in each chapter assumes 12 groups of 2 or 3 students. If you have more or fewer lab groups, adjust the amount of materials accordingly. The information below will assist you in these adjustments.

NOTE: Some labs require preparation several days before the lab, so read through the *Preparation* section for each chapter you plan to use before beginning the program.

NOTE: The preparation time required for the ABE labs is significant. You might ask students who have an interest in acquiring lab skills to assist you with preparation.

MATERIALS NEEDED PER GROUP

The following tables include only those materials for which you will need to adjust amounts depending on how many groups of students you have in each class.

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 1, Session 1, Laboratory 1.1	Additional preparation and materials for Laboratory 1.1	Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with microfuge tube of red dye solution (RD)	1
			b. P-20 micropipette	1
			c. Tip box of disposable pipette tips	1
			d. Laminated micropipette practice sheet (from kit)	1
			e. Waste container	1 for every two groups
Chapter 1, Session 2, Laboratory 1.2	Make agarose gels for Laboratory 1.2[2] (can be done several days in advance)	Prepare the agarose solution:	a. 20x SB	1.5 mL
			b. Distilled water (dH_2O)	28.5 mL
			c. Agarose	0.24 g
			d. Sandwich- or quart-sized resealable bags	1
	Additional preparation and materials for Laboratory 1.2	Prepare 1x SB *(can be done several days in advance)*:	a. 20x SB	2.5 mL
			b. dH_2O	47.5 mL
			c. 50-mL flask labeled "1x SB"	1 for every two groups
		Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of red dye (RD) ii. Microfuge tube of dye solution 1 (S1) iii. Microfuge tube of dye solution 2 (S2) iv. Microfuge tube of dye solution 3 (S3)	1
			b. P-20 micropipette	1
			c. Tip box of disposable pipette tips	1
			d. Pipetting practice plates loaded with 0.8% agarose gel	2
			e. Waste container	1 for every two groups

[2] Amounts of materials listed will make one gel, which can be used by two groups.

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 1, Session 3, Laboratory 1.3 (Optional)	Additional preparation and materials for optional Laboratory 1.3	Copy Reproducible Master 1 and gather the following materials:	a. **Laboratory 1.3: Examining Micropipette Precision (RM 1)**	1 copy (per student)
			b. Plastic microfuge tube rack with a microfuge tube of dH_2O	1
			c. P-20 micropipette	1
			d. Tip box of disposable pipette tips	1
			e. 1.5-mL microfuge tubes	8
			f. Permanent marker	1
			g. Medicine dropper	1
			h. Waste container	1 for every two groups

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 2A, Session 2	Copy handouts and gather materials for ***Clone That Gene***	Copy the Reproducible Masters and gather the following materials:	a. **Plasmid Diagram (RM 2)**	1 copy (per pair)
			b. **Human DNA Sequence (RM 3)**	1 copy (per pair)
			c. Scissors	1 (per pair)
			d. Tape	1 roll (per pair)
Chapter 2A, Session 2, Laboratory 2A	Aliquot reagents for Laboratory 2A *(can be done several days in advance)*	Label four 1.5-mL microfuge tubes as follows:	a. Microfuge tube marked "2.5xB"	1
			b. Microfuge tube marked "PR"	1
			c. Microfuge tube marked "RE"	1
			d. Microfuge tube marked "dH_2O"	1
		Pipette reagents into the microfuge tubes as follows:	a. 2.5x restriction buffer into tube marked "2.5xB"	12.0 µL
			b. pARA-R plasmid solution into tube marked "RP"	10.0 µL
			c. Restriction enzymes into tube marked "RE"	3.0 µL
			d. Distilled water into tube marked "dH_2O"	1,000 µL
	Gather materials for Laboratory 2A	Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of 2.5xB ii. Microfuge tube of RP iii. Microfuge tube of RE iv. Microfuge tube of dH_2O	1
			b. P-20 micropipette	1
			c. Tip box of disposable pipette tips	1
			d. Disposable gloves	1 pair (per student)
			e. 1.5-mL microfuge tubes	2
			f. Permanent marker	1
			g. Waste container	1 for every two groups

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 4A, Sessions 1 and 2, Laboratory 4A	Make agarose gels for Laboratory 4A[3] *(can be made several days in advance)*	Prepare the agarose solution:	a. 20x SB	1.5 mL
			b. dH_2O	28.5 mL
			c. Agarose	0.24 g
			d. Sandwich- or quart-sized resealable bags	1
	Aliquot reagents for Laboratory 4A *(can be done several days in advance)*	Label two 1.5 mL microfuge tubes as follows:	a. Microfuge tube marked "LD"	1
			b. Microfuge tube marked "M"	1
		Pipette reagents into the microfuge tubes as follows:	a. Loading dye into tube marked "LD"	20.0 µL
			b. DNA ladder into tube marked "M"	10.0 µL
	Additional preparation and materials for Laboratory 4A	Prepare 1x SB *(can be done several days in advance)*:	a. 20x SB	2.5 mL
			b. dH_2O	47.5 mL
			c. 50-mL flask labeled "1x SB"	1 for every two groups
		Copy the Reproducible Master and prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of digested pARA-R from Laboratory 2A (R+) ii. Microfuge tube of nondigested pARA-R from Laboratory 2A (R–) iii. Microfuge tube of LD iv. Microfuge tube of M	1
			b. P-20 micropipette	1
			c. Tip box of disposable pipette tips	1
			d. Waste container	1 for every two groups
			e. **DNA Ladder Diagram (RM 4A)**	1 copy (per student)

[3] Amounts of materials listed will make one gel, which can be used by two groups.

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 5A, Sessions 1–3, Laboratory 5A	Aliquot reagents and gather materials for Laboratory 5A *(can be done several days in advance)*	Label three 1.5-mL microfuge tubes as follows:	a. microfuge tube marked "LB"	1
			b. Microfuge tube marked "RP"	1
			c. Microfuge tube marked "CC"	1
		Pipette reagents into the microfuge tubes as follows:	a. Luria Broth into tube marked "LB"	350 µL
			b. pARA-R recombinant plasmid into tube marked "RP"	12.0 µL
		Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of LB ii. Microfuge tube of RP	1
			b. 1.5-mL microfuge tubes	2
			c. Permanent marker	1
			d. Disposable gloves	1 pair (per student)
			e. P-20 micropipette	1
			f. P-200 micropipette	1
			g. Tip box of disposable pipette tips	1
			h. 3 Petri plates with agar: i. LB plate (one stripe) ii. LB/amp plate (two stripes) iii. LB/amp/ara plate (three stripes)	 1 1 1
	Aliquot reagents and gather materials for Laboratory 5A *(to be done right before lab)*	Copy the Reproducible Master and gather the other materials needed for the lab:	a. Styrofoam cup	1
			b. Cell spreaders	2
			c. Colored tape	1 roll for every two groups
			d. Biohazard bag (for materials that come into contact with *E. coli* cells)	1 for every two groups
			e. Liquid waste collection container, such as a small beaker	1 for every two groups
			f. **Bacterial Growth Predictions (RM 5)**	1 copy (per student)
		Prepare competent cells 15 minutes before students begin the lab:	a. Iced tube labeled "CC"	1
			b. Competent *E. coli* cells	100 µL

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 6, Session 2, Laboratory 6, Part A	Aliquot reagents for Laboratory 6, Part A *(can be done a day or two before the lab)*	Label three 1.5-mL microfuge tubes as follows:	a. Microfuge tube marked "EB"	1
			b. Microfuge tubes marked "LyB"	1
			c. Microfuge tube marked "EC"	1
		Pipette reagents into the microfuge tubes as follows:	a. Elution buffer into tube marked "EB"	200 µL
			b. Lysis buffer into each tube marked "LyB"	160 µL
		Pipette LB/amp/ara suspension culture of *E. coli* into microfuge tube as follows:	a. LB/amp/ara suspension culture of *E. coli*	1,000 µL (1 mL)
	Gather materials for Laboratory 6, Part A	Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of *E. coli* (EC) ii. Microfuge tube of EB iii. Microfuge tube of LyB	1
			b. Liquid waste collection container, such as a small beaker	1
			c. P-200 micropipette	1
			d. Tip box of disposable pipette tips	1
			e. Permanent marker	1
			f. Biohazard bag (for materials that come into contact with *E. coli* cells)	1 for every two groups

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 6, Session 2, Laboratory 6, Part B	Gather materials for Laboratory 6, Part B	Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with EC microfuge tube from Part A	1
			b. The following reagents:[4] i. Binding buffer (BB) ii. Wash buffer (WB) iii. Elution buffer (EB) iv. Column equilibration buffer (CEB)	 1 1 1 1
			c. 1.5-mL microfuge tubes	2
			d. Chromatography column	1
			e. Liquid waste collection container, such as a small beaker	1
			f. P-1,000 micropipette	1
			g. Tip box of disposable pipette tips	1

ALIQUOTING REAGENTS, DNA, AND ENZYMES

The ABE lab protocols have been written to minimize the amount of aliquoting that you must do; it has not, however, been eliminated entirely. Guidelines for aliquoting are included for each lab in the ***Preparation*** section. Where appropriate, suggestions are made that may save aliquoting time. For example, rather than make a single aliquot for each group, there are times when a double aliquot can be made and two groups can share the reagent.

SOLUTION PREPARATION

Most of the solutions in your kit are at working concentrations so that you will not have to dilute them before aliquoting to students. However, some may be sent to you at higher concentrations (that is, "stock" concentration) than the concentrations students actually use. For example, the sodium borate buffer (SB buffer, for electrophoresis) is provided at a stock concentration of 20x; students

[4] You will be provided with either 15-mL tubes of each buffer or one large container of each buffer. If you have the 15-mL tubes, one can be given to each group. If you have the larger container, pour 10 mL of each buffer into a set of flasks that can be shared by two groups.

will only work with the SB buffer at 1x concentration. Diluting a large volume of the SB buffer to the working concentration will save you preparation time.

> **NOTE: Once used, 1x SB buffer can remain in the electrophoresis box or discarded. If you have back-to-back classes, keep the buffer in the electrophoresis boxes for classes doing the same lab.**

To calculate the dilution from a stock concentration to a working concentration:

1. Determine how much working solution you need per group.
2. Multiply the volume needed per group by the number of groups.
3. Use the formula $C_1V_1 = C_2V_2$ to determine the volume of stock solution (V_1) at the stock concentration (C_1) you will need to dilute in order to prepare the final volume of working solution (V_2) at the working concentration (C_2).
4. Multiply this volume by the number of classes you have.

Example: You need to prepare 1x SB buffer to make agarose gels. If each of your classes has 12 lab groups, you will need six gels for each class. Each gel needs approximately 30 mL of 1x SB buffer. The SB buffer supplied has a concentration of 20x. You have five classes conducting the ABE labs.

- *Determine how much solution of working concentration you will need per group:*

  30 mL of 1x SB buffer per gel

- *Multiply the volume needed per group by the number of groups:*

  30 mL × 6 gels = 180 mL

- *Use the formula $C_1V_1 = C_2V_2$:*

  20x × V1 = 1x × 180 mL

  V1 = (1x × 180 mL) / 20x

  V1 = 9 mL of 20x SB buffer

  You need to mix 9 mL of 20x SB buffer and 171 mL of dH_2O (deionized or distilled) to make 180 mL of 1x SB buffer for one class.

- *Multiply this volume by the number of classes you have:*

  5 x 9 mL = 45 mL

  Since you have five classes, you need to mix 45 mL of 20x SB buffer and 855 mL of dH_2O. This will make 900 mL of 1x SB buffer, enough for all five classes.

ADDITIONAL TEACHING INFORMATION

Just as students need help in conducting experiments and interpreting results, they need support in making use of the literacy tools that are an essential part of scientific inquiry. Whatever their proficiency in reading, students encountering scientific texts often need help to make sense of what they are reading and to learn scientific concepts. Each chapter has one or more readings that serve different functions: to engage students by relating what they are about to learn to real-world situations, to provide the background information needed to complete an activity, or to summarize the conceptual understandings that students should have acquired.

You can use a number of strategies to support students in the reading of scientific material. Some of the readings can be read out loud during class time, and some can be completed as homework. In either case, the students' reading should be followed by a series of questions (who, what, why, when, and where) to be discussed in a whole-class setting. Writing the answers on chart paper with a drawing related to the main idea and then posting them for reference can also be effective. Students may build their own glossary, using their own words to define unknown vocabulary. They can identify words they don't know, bring the words into a whole-class discussion, and add them to their glossary.

LEADING CLASSROOM DISCUSSIONS

Class discussions are an integral strategy for teaching and learning in the ABE program:

- Questions that ask students to analyze an experiment or review a reading, either on their own or as part of a group, can be used to initiate a discussion. As the discussion moves forward, students may raise their own questions.
- Whole-class discussion gives students the opportunity to relate real-life experiences that they might have had concerning the topic of discussion, which makes the subject authentic, practical, and relevant.
- Discussion can be used to brainstorm ideas. Students then have a chance to explain their ideas and provide evidence for their conclusions.
- Discussions requiring prior knowledge may reveal preconceptions about a topic.
- The process of critiquing explanations in a public forum enables students to reach a greater depth of understanding and to confront certain preconceptions.
- Discussions can be used to inform instruction by enabling you to assess prior understanding, determine the level of student understanding of the concepts being discussed, and determine students' logic and reasoning.

Students may not be accustomed to discussions in science classrooms. Encouraging all students to participate and to practice "wait time" will increase

class engagement and the quality of the responses. Allowing students to have small-group discussions before entering into whole-class discussions will give more reticent students the opportunity to gain confidence in speaking before a group and to learn the value of their contributions.

As facilitator, you have several tasks:

- Pose thought-provoking questions.
- Break larger questions into smaller, more manageable ones.
- Help students clarify their thinking by rephrasing or asking different questions.
- Keep the discussion focused on the concepts being explored.
- Help students practice discussion "etiquette" in listening and responding to others.

LAB NOTEBOOKS AND REPORTS

Students should each use a lab notebook that can serve as a repository of all their work, including responses to questions answered individually or as part of a group, notes taken during discussions, data and comments recorded in laboratory investigations, and writing assignments. This notebook will enable students to refer back to their ideas and thinking, and allow both you and your students to assess their progress in conceptual understanding and to monitor improvements in skills such as writing, data collection, critical analysis, and thinking.

As students engage in the ABE program, give them the opportunity to write lab reports that follow a sequence of creating a hypothesis, collecting and analyzing data, and making conclusions and claims that are supported by evidence.

USING FLOWCHARTS IN THE LAB

Given the complexity of some of the ABE lab protocols, it will be very difficult for students to complete the laboratories on time and correctly if they do not review the protocols in advance. In addition, given that there are many steps to each protocol, students should develop a flowchart to work from during each lab. Students should develop flowcharts that are detailed enough that they do not need to refer to the Student Guide during the lab and that allow space for them to record notes during the experiment.

Sample flowcharts are provided for each laboratory and as Reproducible Masters on the program website. For the first few labs, you might want to provide students with a sample—either fully or partially completed.

Check each student's flowchart before he or she begins the laboratory.

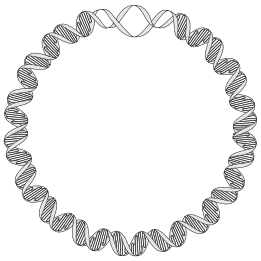
RESOURCES

Links to resources that you can use to augment student learning are available on the program website. These resources include videos, articles related to the content of the curriculum, and labs that can be used to extend student learning.

ICONS

Icons are used throughout the Teacher and Student Guides to draw attention to various aspects of the curriculum. The following is a list of those icons and their meanings.

Icon	Meaning	Student Guide	Teacher Guide
	**DID YOU KNOW?**: Background information about concepts covered in the chapter.	✓	
	**STOP AND THINK**: Key questions for improving student understanding of the lab protocols.	✓	✓
	**CONSIDER**: Key questions for improving student understanding of important biological concepts.	✓	✓
	**SAFETY**: Reminders of key lab safety techniques.	✓	✓
	**LAB TECHNIQUE:** Useful lab techniques to improve efficiency and results.	✓	✓
	**KEY IDEAS**: Important concepts that are central to each session.		✓
	**RESOURCES**: Information about resources found on the program website.		✓
	**GOING BEYOND**: Suggested areas for expanding beyond the curriculum.		✓
	**STRATEGY**: Suggestions for best practices while teaching this content.		✓



PROGRAM INTRODUCTION

AMGEN BIOTECH EXPERIENCE

OVERVIEW

The Program Introduction provides a context for the lab experiences your students will have in the Amgen Biotech Experience (ABE). Students read about genetic engineering and biopharmaceutical (biopharma) research.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- The relationship between DNA, genes, proteins, and traits—specifically, that genes contain the code for making a protein and that proteins are molecules that are used in making and running the cell, so they are responsible for traits

LEARNING GOALS

By the end of the Program Introduction, students will be able to do the following:

- Explain that genetic engineering creates genetically modified organisms that make human proteins from human DNA
- Explain how genetic engineering can be used to treat some diseases

ASSESSED OUTCOME

- Assess each student's ability to explain that genetic engineering creates genetically modified organisms that make human proteins from human DNA and how genetic engineering can be used to treat some diseases by reviewing students' work on the K-W-L and the Word Wall in the second session.

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Have students begin a K-W-L on the concepts of biotechnology and the biopharmaceutical industry. (10 min.)
- Have students read **What Is Biotechnology?** Lead a discussion on the reading. (35 min.)

SESSION 2

- Have students complete the K-W-L on the concepts of biotechnology and the biopharmaceutical industry. (20 min.)
- Have students create a Word Wall defining the words in **What Is Biotechnology?** (20 min.)
- Have students record one question related to the content of **What Is Biotechnology?** (5 min.)

PREPARATION

Familiarize yourself with the content in the Curriculum Overview and in this introduction. Choose and bookmark a video that features discussions of biotechnology and its contributions.

MATERIALS FOR READING ACTIVITIES

Gather the following materials and distribute them to students as needed:

- Three sheets of newsprint, labeled "Know (K)," "Want to Know (W)," and "Learned (L)," respectively
- Markers
- Highlighters or sticky notes for note-taking
- One index card or sticky note per student
- Scotch or masking tape

TEACHING

SESSION 1

KEY IDEAS: Genetic engineering allows humans to insert human DNA into other organisms and then have these genetically-modified organisms make human proteins. These proteins can be used to treat a wide variety of diseases and help millions of people.

Have students begin a K-W-L on the concepts of biotechnology and the biopharmaceutical industry. (10 min.)

Post the prepared sheets of newsprint—"Know (K)," "Want to Know (W)," and "Learned (L)." Ask students to indicate what they already know about biotechnology ("K") and what they want to know ("W"), and note this on the newsprint. Keep the sheets posted in the classroom during the program.

STRATEGY: After each lab and/or reading, revisit the "W" sheet and begin adding to the "L" sheet. Revisit the "W" and "L" sheets at the end of the program to assess how students' knowledge about this field has evolved.

Have students read **What Is Biotechnology?** Lead a discussion on the reading. (35 min.)

In this reading, students learn that biotechnology began after the discovery of plasmids and restriction enzymes and that it is used to develop products and

technologies that improve human health, create fuels to power the world, and develop better systems for the production of food. Remind students to use the ***Glossary*** to look up scientific terms if they need help understanding the reading.

Show one or more videos that feature discussions of biotechnology and its contributions. After viewing the video(s), ask students to brainstorm ideas about the future and promise of biotechnology.

RESOURCES: Links to videos you might show students are available on the program website.

To help students comprehend the reading and video(s), have them work in groups of two to four and carry out the following reading strategies:

- Highlight or record on sticky notes any words and concepts they think are important as they read.
- Record questions they have about the reading or related content.

Tell students that they will share some of their notes and questions in the following class session.

STRATEGY: As you lead the discussion, use the following practices and techniques:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

SESSION 2

KEY IDEAS: Biopharma researchers study human biology to better understand how to develop solutions to improve the lives of people who suffer from serious diseases.

Have students complete the K-W-L on the concepts of biotechnology and the biopharmaceutical industry. (20 min.)

Ask students to revisit the "W" sheet and begin adding to the "L" sheet, using what they learned from **What Is Biotechnology?** Explain that students will do activities and labs over the next few days that will give them greater insight into biotechnology and biopharma.

STRATEGY: If students need additional support in understanding the reading, lead a class discussion using the following questions:

- **What important idea did you take away from this reading?**
- **How do you think the information in this introduction relates to the labs you are about to conduct?**

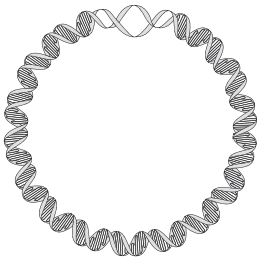
Have students create a Word Wall defining the content words in **What Is Genetic Engineering?** (20 min.)

Hand out one index card or sticky note and one marker to each student and ask students to write down a key word or term from the reading. Have students post the cards one at a time, grouping the same or similar words.

STRATEGY: You may want to keep this "Word Wall" posted and revisit it periodically—you can add to it throughout the program, but limit the number of words to 10–20. Encourage students to refer to the Word Wall during class discussions or as they are reading lab protocols. You can also use the Word Wall as a tool to assess student learning by asking students to develop concept maps showing the relationships among the words.

Have students record one question related to the content of **What Is Biotechnology?** (5 min.)

STRATEGY: You may want to want to revisit these questions at the end of the program (as a class or individually) to assess students' understanding of the content.



CHAPTER 1

SOME TOOLS OF THE TRADE

OVERVIEW

Chapter 1 introduces students to some tools of genetic engineering, and the associated labs give students hands-on experience with some important tools and techniques commonly used in molecular biology. Students are also introduced to some volumetric measurements that are most often used in this field of science.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- The relationship between DNA, genes, proteins, and traits—specifically, that genes contain the code for making a protein and that proteins are molecules that are used in making and running the cell, so they are responsible for traits
- That charged objects, including molecules, move through an electric field

LEARNING GOALS

By the end of this chapter, students will be able to do the following:

- Correctly use micropipettes and the technique of gel electrophoresis
- Explain the importance of micropipettes and gel electrophoresis in genetic engineering
- Describe how gel electrophoresis separates DNA
- Explain how genetic engineering can be used to treat some genetic diseases

ASSESSED OUTCOMES

- Assess each student's ability to use micropipettes and the technique of gel electrophoresis by reviewing their work in Laboratories 1.1 and 1.2, and by reviewing their responses to the *STOP AND THINK* questions in Laboratory 1.1 (page 19 of the Student Guide) and to the first *STOP AND THINK* question in Laboratory 1.2, Part B (page 24 of the Student Guide).
- Assess each student's ability to explain the importance of micropipettes and gel electrophoresis in genetic engineering by reviewing their responses to question 1 in *Before the Lab* in Laboratory 1.1 (page 17 of the Student Guide,) to question 1 in *Before the Lab* in Laboratory 1.2 (page 21 of the Student Guide), and to question 1 in *Chapter 1 Questions* (page 26 of the Student Guide).
- Assess each student's ability to describe how gel electrophoresis separates DNA by reviewing their responses to the second and third *STOP AND THINK* questions in Laboratory 1.2, Part B (page 24 of the Student Guide).

- Assess each student's ability to explain how genetic engineering can be used to treat some genetic diseases by reviewing their responses to question 2 in *Chapter 1 Questions* (page 26 of the Student Guide).

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Review the **Introduction** and *Chapter 1 Goals*. (5 min.)
- Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)
- Have students complete Laboratory 1.1. During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions with the class and explain their thinking. (20 min.)
- Have students read the introduction to Laboratory 1.2 and answer the *Before the Lab* questions. (10 min.)

SESSION 2

- Have students complete Laboratory 1.2. During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions with the class and explain their thinking. (35 min.)
- Have students discuss *Chapter 1 Questions* in small groups and record their answers individually. Lead a discussion on students' answers. (10 min.)

SESSION 3 (OPTIONAL)

- Have students complete **Laboratory 1.3 (RM 1**). During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions with the class and explain their thinking. (45 min.)

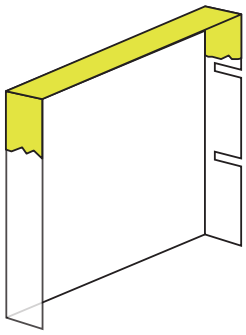
PREPARATION

Before you begin, you should become familiar with the laboratory procedures in this chapter, the preparation required, and the materials you'll need. The instructions assume that you will provide materials for 12 groups of 2 or 3 students. Multiply the amounts as necessary depending on the number of students and number of classes you are teaching.

MAKE AGAROSE GELS FOR LABORATORY 1.2

RESOURCES: The video *Making an Agarose Gel* (available on the program website) goes through the process of making and casting an agarose gel, as described below.

1. Gather the following materials:
   - 6 gel electrophoresis trays
   - 6 10-well combs
   - Optional: Tape



2. Set out the 6 electrophoresis gel trays and 6 combs. Prepare the trays for casting by securing the gates on the ends of each tray in the "up" position or taping the ends of each tray. Place a comb in each tray before adding the agarose solution.

3. Prepare the agarose solution.

   a. Gather the following materials:
      - 2 250-mL graduated flasks, one labeled "1x SB buffer"
      - 500-mL flask labeled "Gel"
      - 12.5 mL of 20x sodium borate buffer (20x SB buffer)
      - 237.5 mL of distilled or deionized water
      - 1.44 g of agarose
      - Mass scale
      - Plastic wrap
      - Disposable pipette tip
      - Microwave
      - Heat-resistant gloves or tongs
      - 6 sandwich- or quart-sized resealable bags
      - Waste container for used tips and microfuge tubes

   b. Prepare 250 mL of 1x sodium borate buffer (1x SB buffer). Add 12.5 mL of 20x SB buffer to the 250-mL graduated flask labeled "SB buffer," add distilled or deionized water to the 250-mL mark, and mix.

   c. Pour 180 mL of 1x SB buffer into the second 250-mL graduated flask.

d. Measure 1.44 g of agarose with the mass scale and place it in the 500-mL flask labeled "Gel." Add the 180 mL of 1x SB buffer from step 3c to make 0.8% agarose solution.

e. Cover the opening of the 500-mL flask with plastic wrap. Use the pipette tip to poke a small hole in the plastic wrap.

f. Place the covered flask in a microwave and heat for one minute on high. With a gloved hand, gently swirl the flask. (Alternatively, a hot plate can be used to melt the agarose, but you will need to use a double boiler.)

SAFETY: Wear heat-resistant gloves or use tongs to hold the flask.

g. Continue microwaving the flask for 5–15-second intervals until all the agarose has dissolved. To check this, hold the flask to the light and swirl the solution. Look carefully for "lenses" of agarose crystals suspended in the liquid. If no lenses are visible, the agarose is dissolved. Wait five minutes for the agarose to cool to about 60 °C before continuing to step 4.

LAB TECHNIQUE: Try not to allow the solution to cool to the point that the agarose begins to re-solidify—but if it does, simply reheat the solution as described above.

4. Cast the gels in the prepared trays.

When the agarose solution has cooled to the point that you can safely touch the bottom of the flask (approximately 60°C; this will take around five minutes), pour 25–30 mL of the agarose solution into each electrophoresis tray.

a. Be sure to include the combs when casting the gels. The solution should cover about 2 mm of each comb.

b. Once the gels solidify (which will take around 30 minutes), pull the comb out of each gel. Pull it straight out without wiggling it back and forth; this will minimize damage to the front wall of the well.

c. Remove the gels from the gel electrophoresis trays and store them in individual resealable bags with a small amount of the remaining 1x SB buffer from step 3b. Store in the refrigerator until ready to use. Be sure to keep them flat and not on a textured surface, as textured surfaces will imprint onto the gels and impact how molecules move through them.

ADDITIONAL PREPARATION AND MATERIALS FOR LABORATORY 1.1

Prepare 12 sets of materials that each include the following:

1. Plastic microfuge tube rack that contains a microfuge tube of red dye solution (RD, from kit)
2. P-20 micropipette
3. Tip box of disposable pipette tips
4. Laminated micropipette practice sheet (from kit)
5. Waste container for used tips and microfuge tubes (one container for every two groups)

ADDITIONAL PREPARATION AND MATERIALS FOR LABORATORY 1.2

1. Prepare 300 mL of 1x SB buffer.

   LAB TECHNIQUE: You should prepare 1x SB buffer for all classes that will complete this lab—simply multiply the quantities given by the number of classes.

   a. Gather the following materials:

      - 15 mL of 20x SB buffer
      - 500-mL graduated flask labeled "SB buffer"
      - 285 mL of distilled or deionized water
      - 6 50-mL flasks labeled "SB buffer"

   b. Add 15 mL of 20x SB buffer to the 500-mL flask labeled "SB buffer," add distilled or deionized water to the 300-mL mark, and mix.

   c. Pour 50 mL of SB buffer into each of the 50-mL flasks labeled "SB buffer."

2. From your kit, gather 12 sets of four tubes (one each of RD, S1, S2, and S3). Be sure to return these tubes to the kit after the lab.

3. Prepare 12 sets of materials that each include the following:

   - Plastic microfuge tube rack that contains the following reagents:
     - Microfuge tube of red dye (RD)
     - Microfuge tube of dye solution 1 (S1)
     - Microfuge tube of dye solution 2 (S2)
     - Microfuge tube of dye solution 3 (S3)
   - P-20 micropipette
   - Tip box of disposable pipette tips
   - 2 pipetting practice plates loaded with 0.8% agarose gel
   - Waste container for used tips and microfuge tubes (one container for every two groups)

4. Set up six electrophoresis boxes, each near a power supply; two groups will share one box. Once boxes are set up, load each one with an agarose gel (prepared above) and set one 50-mL flask containing 1x SB buffer (prepared above) near each box.

5. Put the microcentrifuge in a central location so that all groups can share it.

ADDITIONAL PREPARATION AND MATERIALS FOR THE OPTIONAL LABORATORY 1.3

Materials for students are listed on **Laboratory 1.3: Examining Micropipette Precision (RM 1)**, found at the end of this guide.

1. Make one copy of **Laboratory 1.3: Examining Micropipette Precision (RM 1)** for each student.

2. Gather 12 1.5-mL microfuge tubes and label them "dH_2O."

3. Using a P-200 micropipette, dispense 200 µL of distilled water into the tubes marked "dH_2O."

4. Prepare 12 sets of materials that each include the following:
   - Plastic microfuge tube rack that contains a microfuge tube of dH_2O (prepared in step 3)
   - P-20 micropipette
   - Tip box of disposable pipette tips
   - 8 empty 1.5-mL microfuge tubes
   - Permanent marker
   - Medicine dropper
   - Waste container for used tips and microfuge tubes (one container for every two groups)

5. Put the microcentrifuge in a central location so that all groups can share it.

TEACHING

SESSION 1

KEY IDEAS: Biotechnology requires using very specific tools and having well-honed laboratory skills. Micropipettes are used to measure and transfer very small volumes of liquids.

Review the **Introduction** and *Chapter 1 Goals* with students. (5 min.)

The **Introduction** explains the main purpose of this chapter, linking it to the Program Introduction. The *Chapter 1 Goals* tell students what they should focus on learning as they work through this chapter. Explain to students what you will assess in this chapter and what your expectations are for students' performance.

Have students answer the *What Do you Already Know?* questions and share their responses. (10 min.)

The *What Do You Already Know?* section activates students' knowledge of biotechnology and how precision is necessary for carrying out biotechnology experiments. Have students work in pairs to answer the questions, and record their ideas; this will help them assess what they know and don't know about biotechnology and the experimental procedures used in it.

NOTE: The level of students' answers to these questions may vary a great deal according to their prior knowledge. For example, students in higher-level biology classes will likely have a much more extensive knowledge of different biotechnological tools and techniques and a deeper understanding of why precision is necessary.

Possible answers to the *What Do You Already Know?* questions:

1. What tools and techniques of biotechnology have you used before? What did you use them for? *Answers will vary greatly according to students' experiences.*

2. Why is precision important when you are carrying out biotechnology procedures? *Accuracy and precision of measurements help to ensure that experiments are both successful and reproducible.*

Have students complete Laboratory 1.1. During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions with the class and explain their thinking. (20 min.)

In this lab, students become familiar with how to use the micropipette to load and dispense liquids and they compare the relative size of different amounts measured by a micropipette. Set up a system of materials management for students to follow. Students should be responsible for collecting and returning their kits.

Students discuss the *Before the Lab* questions in their groups and individually record their answers. Have students share their answers with the class.

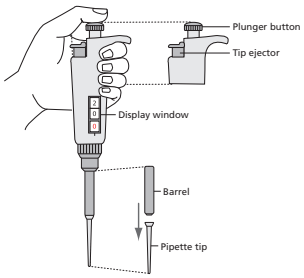
STRATEGY: **For the labs in Chapter 1, you may want to show students the sample flowchart and explain how it can be used to help them recall the steps they should take to complete the lab, rather than have them follow the laboratory *Methods* step by step.**

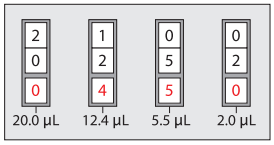
Possible answers to the *Before the Lab* questions:

1. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology? *In this field you would use very small amounts of the reagents and the correct measurements of reagent amounts is necessary for procedures to be successful.*

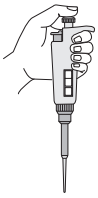
2. Read through the *Methods* section on pages 17 through 19 of the Student Guide and briefly outline the steps, using words and a flowchart. *Students' answers will vary. A student flowchart might look like the one on the following page.*

Laboratory 1.1 Flowchart





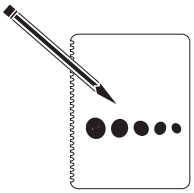
Check reagent (RD) → **Review MP (micropipette)** → **Practice setting MP volume to 20.0 µL, 12.4 µL, 5.5 µL, 2.0 µL** →



Micropipette Practice:

Name	20.0 µL	15.0 µL	10.0 µL	5.0 µL	2.0 µL
1. _____	○	○	○	○	○
2. _____	○	○	○	○	○
3. _____	○	○	○	○	○
4. _____	○	○	○	○	○

Pipette 20.0 µL RD onto laminated sheet → **Pipette other amounts of RD onto laminated sheet** →



Draw sizes of RD amounts in notebook

Before students begin the lab, briefly review the *Methods* section, then review the parts of the micropipette (**Figure 1.1** on page 16 of the Student Guide) and demonstrate how to use it.

RESOURCES: The video *Loading a Pipette* (available on the program website) shows how to use the micropipette.

LAB TECHNIQUE: Review the following with students:

- **Hold the tube at eye level so you can see if the solution is loaded and dispensed properly.**
- **When loading the pipette, always press the plunger to the first stop position *before* immersing the tip in the liquid. This will prevent an air bubble being expelled into the liquid.**

During the lab, students should discuss the *STOP AND THINK* questions in their groups and then individually record their answers. (The *STOP AND THINK* questions help students stay focused on the purpose of the lab.) Have students share their answers and their thinking for each question.

Possible answers to the *STOP AND THINK* questions:

- When loading or dispensing a solution, why is it important to actually see the solution enter or leave the pipette tip? *The amounts are so small and it is easy to become confused and do the steps in the wrong order, so it is important to verify that the solution enters and leaves the tip. If a reagent is missing, the entire experiment will be invalid.*

- You were instructed to avoid contact with the pipette tips—for example, you were asked to put the pipette tip on without using your hands, to avoid setting down the micropipette, to use the ejector button to remove the tip, and to keep the tip box closed. If you were working with plasmids and bacterial cells, why would these precautions be important? *The tips and all surfaces need to remain clean so that the plasmids and bacterial cells do not get contaminated with other substances. You also do not want contaminate yourself or surfaces with bacteria.*

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

Have students read the introduction to Laboratory 1.2 and answer the *Before the Lab* questions. (10 min.)

Students become familiar with the technique of gel electrophoresis. Explain to students that in this lab they will practice using dyes in order to become adept with the technique. If relevant, tell your students that in Chapter 4 or 4A they will use this technique to ensure that they are working with the correct plasmid.

SESSION 2

KEY IDEAS: Those who carry out genetic engineering use very specific tools and have well-honed laboratory skills. Gel electrophoresis allows for the visualization of minute amounts of DNA. Using this technique, scientists can separate and identify pieces of DNA they are working with.

Have students complete Laboratory 1.2. During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions and explain their thinking. (35 min.)

Have students share their answers to the *Before the Lab* questions with the class.

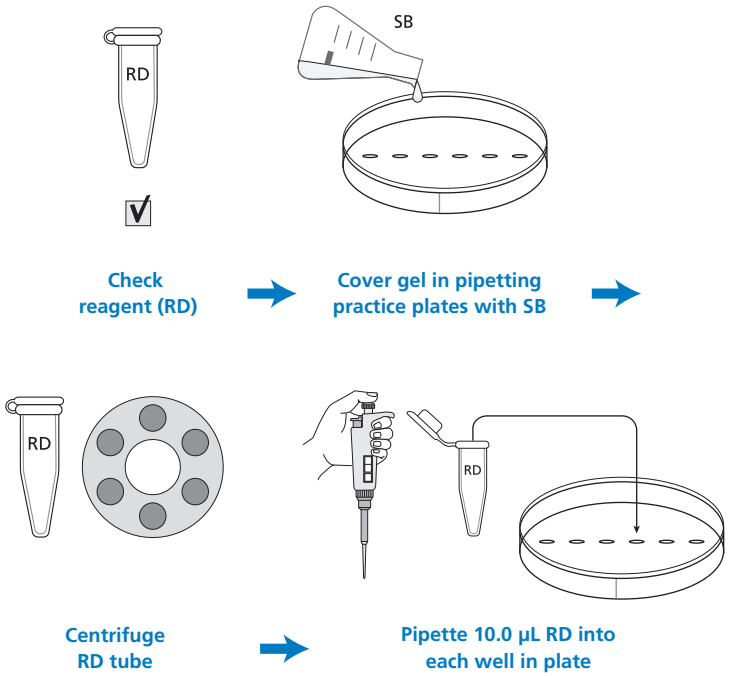
STRATEGY: For the lab, you may want to show students the sample flowcharts on pages 58 and 59 of this guide rather than have them create their own.

Possible answers to the *Before the Lab* questions:

1. In what circumstances might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA? *This would be important if you are making a recombinant plasmid and have to verify that you have been successful.*

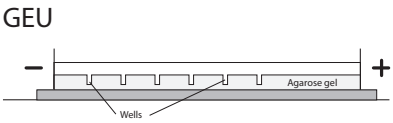
2. Read through the *Methods* section on pages 22 through 25 of the Student Guide and briefly outline the steps for Part A and for Part B, using words and a flowchart. *Students' answers will vary. Their flowcharts might look like the ones on pages 58 and 59 of this guide.*

Laboratory 1.2, Part A Flowchart



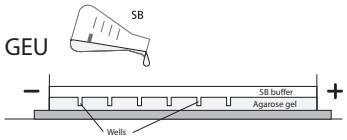
Laboratory 1.2, Part B Flowchart

Check reagents (S1, S2, and S3)

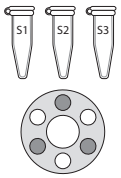


Find out which wells our group will use

Review gel electrophoresis unit (GEU)

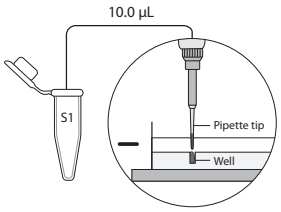


Cover gel in GEU with SB

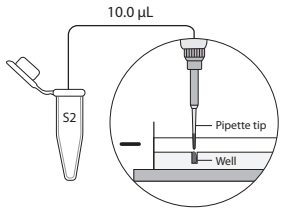


Centrifuge S1, S2, and S3

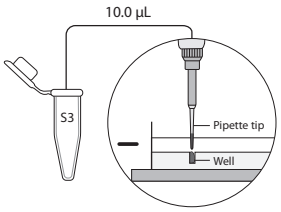
Draw location of each well, and label its solution in notebook



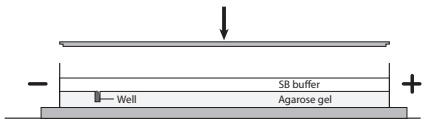
Pipette 10.0 µL S1 into GEU well



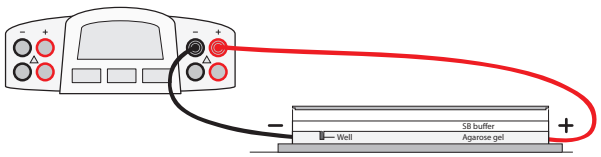
Pipette 10.0 µL S2 into GEU well



Pipette 10.0 µL S3 into GEU well



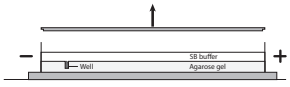
Place cover on GEU



Connect leads to power supply

Turn on power supply and set voltage to 130–135 V

Run gel for 10 minutes



Remove cover from GEU

Draw the relative location of the bands and their colors in notebook

Before students begin the lab, briefly review the *Methods* section, then review the gel electrophoresis unit (**Figure 1.3** on page 20 of the Student Guide) and demonstrate how to use it.

RESOURCES: The video *Working with Small Volumes of Liquid* shows how to mix the liquids with the micropipette, *Using an Electrophoresis Box* shows how to set up the electrophoresis equipment, and *Loading a Gel* shows how to pipette solutions into wells. All three videos are available on the program website.

During the lab, students should discuss the *STOP AND THINK* questions in their groups and then individually record their answers. Have students share their answers and their thinking during the lab with the class.

Possible answers to the *STOP AND THINK* questions:

- Study your gel electrophoresis results. Which solution sample contained a single dye: S1, S2, or S3? How do you know? *S3 contained a single dye because it only had one band.*
- What electrical charge do the dyes have? Explain your reasoning. *The dyes have a negative charge because they are repelled by the negative electrode and attracted to the positive electrode.*
- The dyes that you are separating are orange G (yellow), bromophenol blue (purple), and xylene cyanole (blue). If the molecular shape and electric charge of all three dyes are similar, what is the order of the dyes from heaviest to lightest molecules, based on your initial results? Why do you think this is the correct order? From heaviest to lightest: *Xylene cyanole, bromophenol blue, orange G. This order is based on the appearance of the color bands in the agarose gel after running the electrophoresis box—the blue is closest to the wells, the purple is next closest, and the yellow dye moved the farthest from the wells.*

SCIENCE BACKGROUND: MOVEMENT OF DYES IN GEL ELECTROPHORESIS

The order students may give will likely not be correct, because the dyes used in this lab have different ratios of charge to mass. Although heavier molecules will move more slowly than smaller molecules if both have the same electric charge, molecules with more negative electric charge will move faster than molecules with less negative electric charge if both have the same weight. The molecular weights for the dyes are 408.40 atomic units (au) for orange G, 669.98 au for bromophenol blue, and 538.62 au for xylene cyanole. However, xylene cyanole migrates more slowly than bromophenol blue although it is smaller, because bromophenol blue has a greater charge-to-mass ratio.

The other factor that affects the distance traveled through the gel is shape. Longer or branched molecules will be tangled up in the gel and will move more slowly than shorter or more compact molecules, even if they have the same weight and electric charge. In Chapter 4 students will separate linear pieces of DNA and DNA plasmids. All DNA molecules have the same ratio of charge to mass, so the movement of DNA is determined solely by mass and shape.

GOING BEYOND: You may want to go into more detail regarding the factors that affect distance traveled through the gel than is provided in the Student Guide. You can use the example of the dyes to introduce the idea of charge-to-mass ratio.

Have students discuss the *Chapter 1 Questions* in small groups and record their answers individually. Discuss students' answers as a the class. (10 min.)

Students reflect on the tools they used and their understanding of the genetic engineering process by answering the *Chapter 1 Questions*.

Possible answers to the *Chapter 1 Questions*:

1. Why would it be beneficial to use a micropipette to measure reagents in biotechnology, rather than another measuring instrument? *Micropipettes are used to transfer very small and exact volumes of reagents.*

2. What do the results of gel electrophoresis tell you about genetic material? *Gel electrophoresis is used to separate and identify plasmids and short linear pieces of DNA.*

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

SESSION 3 (OPTIONAL)

KEY IDEAS: The micropipette is far more precise than the medicine dropper.

Have students complete Laboratory 1.3 (RM 1). During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions with the class and explain their thinking. (45 min.)

Have students discuss the *Before the Lab* questions in their groups and then individually record their answers. Have students share their answers to the *Before the Lab* questions with the class.

Possible answers to the *Before the Lab* questions:

1. Why might precision be important in the genetic engineering process? *You need precision in order to mix the correct amounts of reagents together; otherwise, the procedure might not work.*

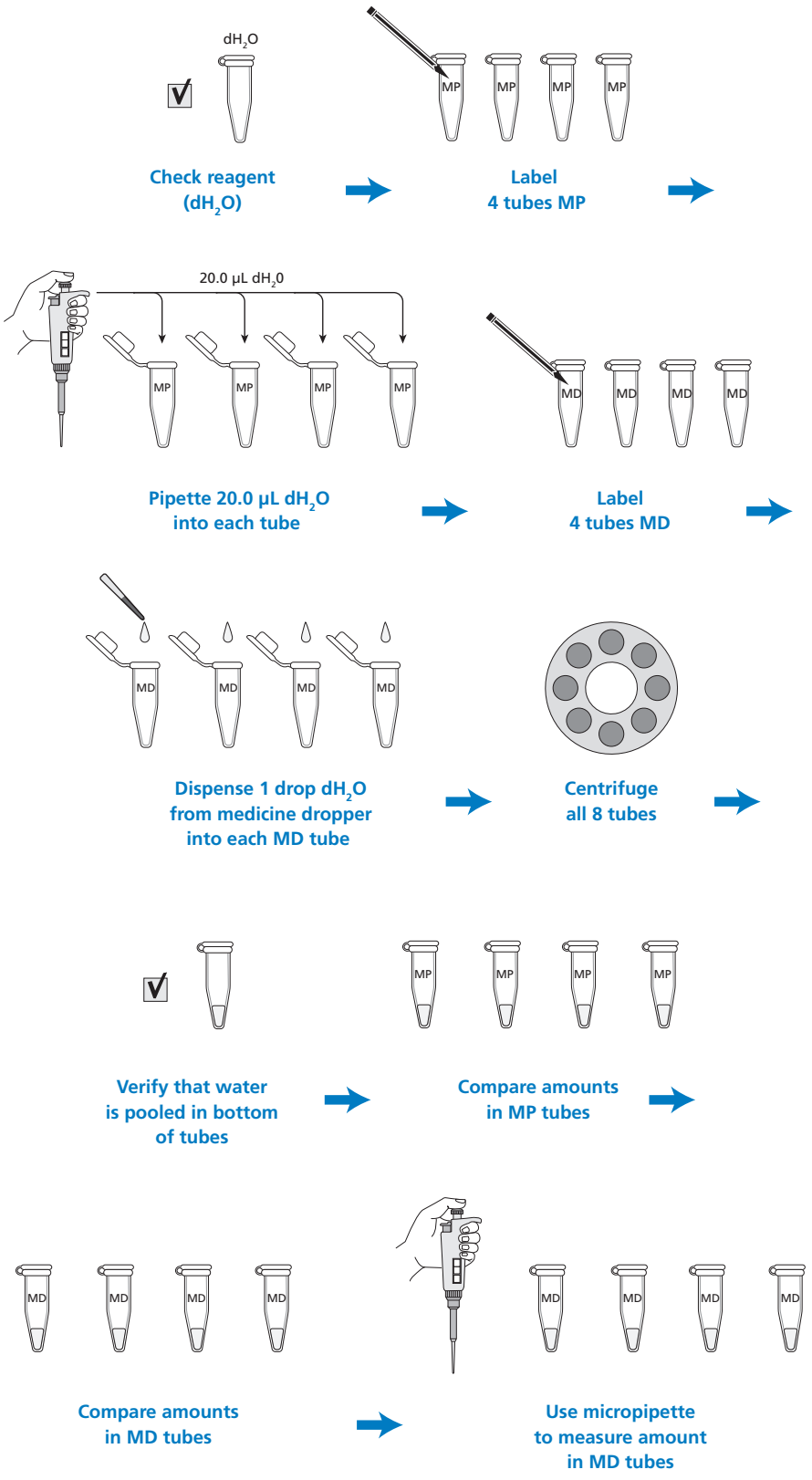
2. Read through the *Methods* section below and briefly outline the steps, using words and a flowchart. *Students' answers will vary. A student flowchart might look like the flowchart on the following page.*

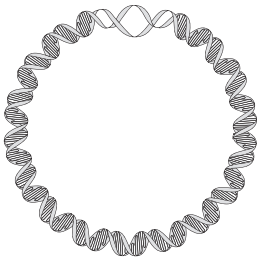
During the lab, students should discuss the *STOP AND THINK* question in their groups and individually record their answers. Have students share both their answers and their thinking about the question.

Possible answer to the *STOP AND THINK* question:

What happens when the water is centrifuged? Why might centrifuging be important when handling small volumes of liquid? *The liquid is consolidated at the bottom of the tube, so it can be more easily dispensed from the tube or more easily mixed if another reagent is added to the tube.*

Laboratory 1.3 Flowchart





CHAPTER 2A

HOW DO YOU BEGIN TO CLONE A GENE?

OVERVIEW

In this chapter students learn about two essential biological tools used in genetic engineering: plasmids and restriction enzymes. Students carry out a paper-based activity in which they determine which restriction enzyme should be used to create a new recombinant plasmid. Students carry out a laboratory activity, Laboratory 2A, in which they use restriction enzymes *Bam*HI and *Hin*dIII to digest the pARA-R plasmid. In preparation for the lab, students describe the DNA fragments that will result from the lab protocol.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- DNA is a double-stranded molecule, and each strand of DNA is made up of covalently linked subunits called nucleotides.
- A nucleotide is made up of a sugar, a phosphate group, and a nitrogenous base. There are four different nitrogenous bases—cytosine, guanine, adenine, and thymine.
- Nucleotides are attached to each other by a sugar-phosphate backbone, while the nitrogenous bases jut out from the backbone.
- The two strands of DNA are connected by hydrogen bonds between adjacent nitrogenous bases, which are called base pairs; cytosine is always paired with guanine, and adenine is always paired with thymine.
- The process of decoding DNA has two steps: a transcription step that transfers the information encoded in DNA into messenger RNA, and a translation step in which the information carried by messenger RNA is used to make proteins.

LEARNING GOALS

By the end of this chapter, students will be able to do the following:

- Describe the characteristics of plasmids
- Explain how plasmids are used in cloning a gene
- Describe the function of restriction enzymes
- Explain how to use restriction enzymes to create a recombinant plasmid

ASSESSED OUTCOMES

- Assess each student's ability to describe plasmid characteristics by reviewing their responses to question 2 in *Activity Questions* (page 42 of the Student Guide) and to questions 1, 3, and 5 in *Chapter 2A Questions* (page 47 of the Student Guide).

- Assess each student's ability to explain how plasmids are used in cloning a gene by reviewing their responses to question 4 in *Chapter 2A Questions* (page 47 of the Student Guide).
- Assess each student's ability to describe the function of restriction enzymes by reviewing their responses to question 2 in *Chapter 2A Questions* (page 47 of the Student Guide).
- Assess each student's ability to explain how to use restriction enzymes to create a recombinant plasmid by reviewing their responses to questions 1 and 3 in *Activity Questions* (page 42 of the Student Guide).

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Review the **Introduction** *and Chapter 2A Goals* with students. (2 min.)
- Have students answer the *What Do You Already Know?* questions and share their responses. (5 min.)
- Introduce and discuss **Your Challenge**. (3 min.)
- Have students read **Beginning to Clone a Gene** and **Producing Human Therapeutic Proteins in Bacteria** and answer the *CONSIDER* questions. (20 min.)
- Lead a discussion on students' answers to the *CONSIDER* questions from **Beginning to Clone a Gene**. (10 min.)
- Introduce the ABE lab sequence the class will follow. (5 min.)

SESSION 2 (OPTIONAL)

- Have students complete one of three optional activities: (1) Carry out Internet research on a pharmaceutical made using a recombinant process, (2) carry out Internet research on a bioethical issue related to genetic engineering and then either have a debate on the issue or write an op-ed or blog post, or (3) extract DNA. (45 min.)

SESSION 3

- Have students carry out ***Clone That Gene*** and discuss the *STOP AND THINK* question as they are working. (25 min.)
- Have students answer the *Activity Questions* in small groups and record their answers individually. (10 min.)
- Lead a discussion on students' answers to all the questions. (10 min.)

SESSION 4

- Have students complete Laboratory 2A. During the lab, have students share their answers to the *Before the Lab* and the *STOP AND THINK* questions and explain their thinking. (25 min.)
- Have students discuss the *Chapter 2A Questions* in small groups and record their answers individually. (10 min.)
- Discuss students' answers as a class. (10 min.)

PREPARATION

Before you begin, you should become familiar with the laboratory procedures in this chapter, the preparation required, and the materials you'll need. The instructions assume that you will provide materials for 12 groups of 2 or 3 students. Multiply the amounts as necessary depending on the number of students and number of classes you are teaching.

LAB TECHNIQUE: Except for the distilled water, all the reagents used in Chapter 2A should be stored in a freezer until you are ready to prepare them for students.

COPY HANDOUTS AND GATHER MATERIALS FOR CLONE THAT GENE

Two handouts are needed for each pair of students—**Plasmid Diagram (RM 2)** and **Human DNA Sequence (RM 3)**. Reproducible Masters (RMs) for the handouts are found at the end of this guide. Obtain a pair of scissors and a roll of tape for each pair. Students will use these materials to construct a paper model of a recombinant plasmid that contains an insulin gene.

ALIQUOT REAGENTS FOR LABORATORY 2A

The reagents can be aliquoted up to several days before Laboratory 2A.

1. Take the following reagents out of the freezer and allow them to defrost for 15 minutes:
   - 2.5x restriction buffer (2.5xB)
   - pARA-R plasmid (RP)
   - Restriction enzymes *Bam*HI and *Hin*dIII (RE)
2. Label microfuge tubes as follows:
   - 12 1.5-mL microfuge tubes marked "2.5xB"
   - 12 1.5-mL microfuge tubes marked "RP"
   - 12 1.5-mL microfuge tubes marked "RE"
   - 12 1.5-mL microfuge tubes marked "dH_2O"

3. Vortex and spin 2.5xB and RE before aliquoting the tubes for student groups. If you do not have a vortex, flick the tube several times to mix and then spin down in the microcentrifuge.

4. Pipette reagents into the microfuge tubes as follows:
   - 12.0 µL of the restriction buffer into the tubes marked "2.5xB"
   - 10.0 µL of the pARA-R plasmid solution into the tubes marked "RP"
   - 3.0 µL of the RE into the tubes marked "RE"
   - 1,000.0 µL of dH_2O into the tubes marked "dH_2O"

5. After aliquoting, store the reagents in the refrigerator until the beginning of the class period in which they will be used.

SET UP AND CALIBRATE WATER BATH FOR LABORATORY 2A

The day before Laboratory 2A, set up and calibrate the water bath to 37°C in order to ensure that the temperature is correct for the incubation of students' restriction digests. It is important that the temperature is not above 37°C, as this will lead to denaturation of the enzyme; err to the low side if necessary. Be sure to use distilled water in the water bath.

1. Gather the following materials:
   - Water bath
   - Thermometer
   - Floating microfuge tube rack
   - Timer

2. Place the water bath in a central location so that all groups can share it.

3. Fill the water bath with distilled water and place the thermometer into it. Warm the water to 37°C, keeping the bath covered to reduce evaporation.

4. Keep the floating microfuge tube rack (for holding the tubes in the water bath) and timer on the table next to the water bath.

5. If classes will conduct Laboratory 5A, leave the water bath set up and calibrate to 42°C the day before the lab. (Be certain that there is sufficient water in the bath and that the bath is covered to reduce evaporation.)

GATHER MATERIALS FOR LABORATORY 2A

Gather materials on the day of the lab. After making up the racks with the reagents, be sure to store them in the refrigerator until students are ready to use.

1. Prepare 12 sets of materials that each include the following:
    - Plastic microfuge tube rack that contains the following reagents (prepared above):
        - Microfuge tube of 2.5xB
        - Microfuge tube of pARA-R plasmid (RP)
        - Microfuge tube of RE
        - Microfuge tube of dH_2O
    - P-20 micropipette
    - Tip box of disposable pipette tips
    - 2 empty 1.5-mL microfuge tubes
    - Permanent marker
    - Waste container for used tips and microfuge tubes (you may only need one container for every two groups)
2. Put the microcentrifuge in a central location so that all groups can share it.

TEACHING

SESSION 1

KEY IDEAS: Plasmids are ideal vectors for use in genetic engineering because they can replicate in the bacteria cell, they have a sequence called a gene promoter that allows a nearby gene to be transcribed and translated, they carry an antibiotic resistance gene that can be used as a selectable marker, and they can be transferred into bacteria by a process called conjugation. The creation of a recombinant plasmid that contains DNA from another species is accomplished by the action of restriction enzymes. Restriction enzymes can cut out a gene of interest from human DNA and can cut the plasmid; the two pieces of DNA can then be joined together. Some restriction enzymes asymmetrically cut DNA at specific sequences, so that the end of one strand overhangs the other end. These ends are called "sticky ends."

Review the **Introduction** and *Chapter 2A Goals* with students. (2 min.)

The **Introduction** explains the main purpose of this chapter, linking it to the Program Introduction. The *Chapter 2A Goals* tell students what they should focus on learning as they work through this chapter. Explain to students what you will assess in this chapter and what your expectations are for students' performance.

Have students answer the *What Do You Already Know?* questions and share their responses. (5 min.)

Answering the questions in this section activates students' knowledge of DNA and how plasmids and restriction enzymes are used in genetic engineering, and reveals the gaps in their knowledge. Have students answer the questions in pairs and record and share their ideas so that you can assess what they know and don't know.

Possible answers to the *What Do You Already Know?* questions:

1. What is the structure and function of DNA? Describe in words or a drawing the structure of a DNA molecule. Be as detailed as possible. *DNA is a double-stranded molecule, and each strand is made up of nucleotides. There are four different nucleotides, which are distinguished by a subpart called a base. The bases are cytosine, guanine, adenine, and thymine. The two strands of DNA are connected by hydrogen bonds between adjacent bases, which are called base pairs. In the base pairs, cytosine is always paired with guanine, and adenine is always paired with thymine. The double strands and the weak hydrogen bonds between base pairs ensure that DNA can be unwound and copied. The four different nucleotides make it possible to create sequences that code for the structures of proteins.*
2. All living organisms contain DNA. In what ways is DNA from different organisms the same, and in what ways does it vary? *All DNA has the same structure and uses the same code and transcription and translation processes. Among different organisms, the DNA sequences will vary because the organisms make different proteins.*
3. Using your understanding of genes and how they are expressed, explain why it is possible for a bacterial cell to make a human protein from the instructions encoded in a human gene. *Because the DNA in all organisms uses the same code and transcription and translation processes, the bacterial cell can create a human protein from a human gene.*
4. Scientists use two biological tools to engineer organisms to make new proteins: plasmids and restriction enzymes. How might each of these be useful in creating a new protein? *Restriction enzymes can cut out a human gene and can cut a plasmid, and these two pieces can be joined together to make a recombinant plasmid that is inserted into bacteria.*

Introduce and discuss **Your Challenge**. (3 min.)

Have students read **Your Challenge** and discuss what they will do in these labs.

Have students read **Beginning to Clone a Gene** and **Producing Human Therapeutic Proteins in Bacteria** and answer the *CONSIDER* questions. (20 min.)

In this reading, students learn why plasmids are an ideal tool for inserting a human gene into bacteria and how restriction enzymes are used to create a recombinant plasmid. Have students record answers to the *CONSIDER* questions in their notebooks. Remind students to use the ***Glossary*** to look up scientific terms if they need help understanding the reading.

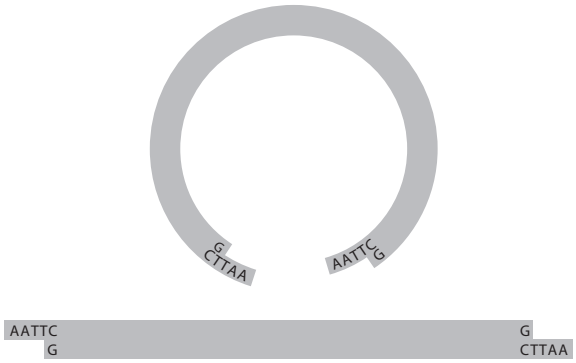
Lead a discussion on answers to the *CONSIDER* questions from **Beginning to Clone a Gene**. (10 min.)

Assess students' knowledge of DNA and how plasmids and restriction enzymes are used in genetic engineering by reviewing students' answers to the *CONSIDER* questions.

Possible answers to the *CONSIDER* questions:

- Use what you know about natural selection and evolution to describe how plasmids might confer a selective advantage to their host bacteria. *If some bacteria carry a plasmid with an antibiotic resistance gene, they will survive when exposed to the antibiotic. This gives them a selective advantage over bacteria that do not carry that plasmid; those bacteria without the plasmid will be killed when exposed to the antibiotic.*
- How do bacteria that carry a restriction enzyme avoid cutting up their own DNA? *The bacteria might have a way to protect their own DNA in sequences where it could be cut by the restriction enzyme, or their DNA might not have the sequence that is cut by the restriction enzyme.*
- What is the sequence of the sticky end that results when DNA is cut with *Bam*HI? With *Hin*dIII? *The overhanging end for* Bam*HI is GATC. The overhanging end for* Hin*dIII is AGCT.*
- Scientists can modify plasmids to have a single restriction enzyme site. Imagine that you have a plasmid with a single *Eco*RI site. Draw the structure of the plasmid after it has been cut with the enzyme, and show the nucleotide sequences left at the site of the cut. If you wanted to insert a

gene from a plant at this site, what enzyme would you use to cut the plant DNA with? Explain your response. *There are two possible ways to represent a plasmid that has been cut with* Eco*RI:*



You would use the Eco*RI restriction enzyme to cut the plant gene. The ends of the gene can then line up with the ends of the plasmid.*

STRATEGY: Students' drawings may vary and you may want to compare different representations. Because a plasmid is a three-dimensional object, students may have trouble visualizing a change to the structure such as a cut. For example, in the second representation of the cut plasmid, the end is flipped around and therefore so is the sequence. If necessary, make paper models for the plasmid and have students carry out the cut on the model.

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

Introduce the ABE lab sequence the class will follow. (5 min.)

Go over the ABE lab sequence that you will be doing. While students may not have the opportunity to complete all the labs, it is important that they know how their work fits into the "big picture" of developing genetically modified organisms to make products that human can use. Review **Figure 2A.4 (**page 39 of the Student Guide), which shows the production of a human therapeutic protein. Point out what parts of the process they will complete in their lab sequence and describe students' specific challenges (see **Table OV.1** on page 11 of this guide).

SCIENCE BACKGROUND: WHY ARE BACTERIAL PROMOTERS IN PLASMIDS?

All genes have their own promoters, so why include a promoter when constructing a plasmid vector? The mechanism of gene transcription is the same in all organisms—RNA polymerase binds to a specific promoter and copies the DNA sequence of the gene into messenger RNA. However, the DNA sequence of promoters and the structure of RNA polymerases may vary; thus, bacterial RNA polymerase will not recognize and bind to a human promoter. Perhaps more importantly, because many eukaryotic genes contain introns and exons, many human genes, such as insulin, that have been cloned are not excised directly from the genomic DNA, but rather DNA is synthesized from the mRNA of the gene of interest by reverse transcriptase. It is this copy DNA (cDNA) that is then cloned. These cDNAs lack a promoter and therefore must be inserted into the plasmid vector near a promoter. For ease of understanding the cloning of the insulin gene, the use of cDNA has been omitted from the reading. You may wish to elaborate on this process with your students.

SESSION 2 (OPTIONAL)

KEY IDEAS: Societal decisions about the implementation of science and technology-related endeavors should be informed both by scientific knowledge and by economics, policies, politics, and ethics. DNA has the same structure and function no matter what organism it comes from.

Have students complete one or more of three optional activities: (1) Carry out Internet research on a pharmaceutical made using a recombinant process, (2) carry out Internet research on a bioethical issue related to genetic engineering and then either have a debate on the issue or write an op-ed or blog post, or (3) extract DNA. (45 min.)

You can extend students' introduction to biotechnology by engaging them in one or more of the activities that follow.

HUMAN THERAPEUTICS NOW AND IN THE FUTURE

Assign students to work individually or in teams to learn more about some of the recombinant products currently in research and development or on the market,

either in this country or abroad, and then present highlights of their findings to the rest of the class. You might suggest that they research what recombinant products are used—or may be used in the future—for common medical conditions, for example:

- Anemia
- Asthma
- Autoimmune diseases, such as lupus or Crohn's disease
- Cancer and/or the side effects of cancer treatments, such as bone marrow transplants and chemotherapy
- Diabetes
- Kidney failure

BIOETHICAL CONSIDERATIONS

There are many potential bioethical issues related to genetic engineering and the biopharmaceutical. Students can research one of the following topics and then either engage in a class debate or write an op-ed or blog post:

- Natural vs. genetically modified insulin: In addition to genetically engineered insulin produced by bacteria, people with diabetes can be treated using insulin taken from cows or pigs. While some people may have adverse reactions to genetically modified synthetic insulin and thus need to take a natural product, others may simply prefer to use natural (as opposed to genetically modified) products. Should their views be respected? Should people with diabetes be allowed to choose?
- Treatment safety vs. treatment access: The invention of new therapeutics, including genetically modified insulin, has saved countless lives. However, before a drug or treatment can be made available to the public, it must be subjected to an extensive battery of tests to determine its effectiveness and to ensure the product is safe for human use. These clinical trials can take many years to complete—time that terminally ill patients often do not have. Patients suffering from life-threatening diseases have long been advocating for access to medications that have not yet completed the entire approval process. They argue that they should be able to receive unapproved medications if they have exhausted all other treatment options. Should their wishes be granted? Is it more important to allow patients access to medications or to ensure that the products are completely safe for human use first?

RESOURCES: Links to news stories about these topics are provided on the program website.

DNA EXTRACTION

At its core, this program is about DNA. DNA codes for proteins, which in turn result in the traits of organisms—whether those traits are fluorescence, reduced insulin production, or brown hair. To help students understand that all DNA has the same structure, have them conduct a DNA extraction lab. Isolating DNA from different organisms and comparing their properties (such as viscosity) will reinforce the idea that no matter what the source, all DNA looks similar and has similar properties.

RESOURCES: Links to possible labs are provided on the program website.

SESSION 3

KEY IDEAS: When creating a recombinant plasmid, it is important to examine the sequences of the plasmid DNA and of the human DNA that contains the gene of interest. It is necessary to find a single restriction enzyme that will cut the plasmid DNA at a single site and will cut near the two ends of the human gene. The identical "sticky ends" created by the cuts from a single restriction enzyme make it possible to join the different sequences of DNA into a recombinant plasmid.

Have students carry out *Clone That Gene*. (15 min.)

Students view genetic sequences of plasmid DNA and a chromosomal target human gene (insulin) and choose the appropriate restriction enzyme to use to create a paper model of a recombinant plasmid. Have students complete this activity in pairs. Note that the insulin gene shown on **RM 3** is a model and is not the full sequence of base pairs in the human insulin gene.

STRATEGY: If several pairs are struggling with the same part of the activity, stop the class and review the instructions for that part. You may want to have students who have successfully completed that part share what they have done.

Have students discuss the *STOP AND THINK* question and the *Activity Questions* in small groups and record their answers individually. (15 min.)

During the activity, students should discuss these questions in their groups and individually record their answers. Have students share their answers and their thinking for the question with the class. Circulate to keep track of their discussions and to provide support.

Possible answer to the *STOP AND THINK* question:

Why is it important that the same enzyme or enzymes be used to cut both the plasmid and the insulin gene from the human DNA? *So that they have*

complementary sequences of bases that can match up and allow the two pieces of DNA to be joined.

Possible answers to the *Activity Questions*:

1. Which restriction enzyme did you choose? Why did you choose that one? *The EcoRI restriction enzyme is the only enzyme that cuts the plasmid once without disrupting a gene.*

2. Where would you insert the insulin gene and why? *The gene should be inserted near the promoter sequence, as this sequence will enable the gene to be transcribed in the bacterial cell.*

3. Which antibiotic would you use to determine if the recombinant DNA was taken in? *Either ampicillin or kanamycin can be used, as both genes are part of the final recombinant plasmid.*

Lead a discussion on students' answers. (15 min.)

Have students share their answers and their thinking for the *STOP AND THINK* and *Activity Questions* with the class. As students share their answers, assess students' knowledge of how restriction enzymes are used in genetic engineering.

SCIENCE BACKGROUND: RESTRICTION ENZYMES

In the early 1970s, Hamilton Smith and Daniel Nathans were able to purify an unknown immune "agent" found in bacteria. This molecular agent protected bacteria by restricting the growth of bacteriophage viruses. The agent was found to be an enzyme that could cut up the viral DNA into fragments as it was injected into its cell. Smith, Nathans, and Werner Arbor received the Nobel Prize for their discovery and characterization of these important molecules.

There are several classes of restriction enzymes, but the ones that have been most useful are those that consistently recognize and cut a specific nucleotide sequence. Some restriction enzymes recognize a four-base sequence; others recognize a five- or six-base sequence. The restriction sites are palindromes. This is an important concept that you may want to emphasize to your students, perhaps using the examples "radar" and "Madam, I'm Adam."

Some restriction enzymes will make a "blunt cut," leaving no overhanging bases. Other enzymes, including *Bam*HI and *Hin*dIII, will leave overhanging bases, thus creating sticky ends. These enzymes are particularly useful since sticky ends make recombining DNA fragments a

fairly simple procedure. The "stickiness" is the result of the extraordinary affinity of complementary nucleotides to form hydrogen bonds between them.

The nomenclature for restriction enzymes is fairly straightforward. The first letter of the enzyme's name is derived from the genus of bacterium from which the enzyme was isolated. The next two letters come from the first two letters of the bacterium's specific epithet. Often there is a fourth letter following the first three, which represents the strain or type of bacterium. Because some strains of bacteria produce several restriction enzymes, a Roman numeral identifies the order in which the enzymes were isolated. **Table 2A.1** on page 36 of the Student Guide shows some examples.

SESSION 4

KEY IDEAS: Scientists who carry out genetic engineering use very specific tools, including tools made by people and biological tools.

Have students complete Laboratory 2A. During the lab, have students share answers to the *Before the Lab* and the *STOP AND THINK* questions and explain their thinking. (25 min.)

Students use restriction enzymes to digest (cut) a plasmid. Explain to students that the restriction enzyme digest will create the fragments that they need to verify that they have the correct recombinant plasmid that will be inserted into bacteria.

NOTES: The following are some important notes for the water bath incubation in this lab:

- **At the end of the lab, load the floating rack with all groups' tubes on the table and then place it in the water bath for a 15-minute incubation.**
- **Leaving the digest in the water bath for a couple of hours will not interfere with the restriction, but the samples should not be left in the water bath for more than two hours, as *Bam*HI can begin to cut DNA randomly.**
- **Following the 15-minute incubation, be sure to place the digest in the freezer until needed for the next lab.**

Students discuss the first *STOP AND THINK* question and the *Before the Lab* questions in their groups and individually record their answers. Have students share their answers with the class.

Possible answer to first *STOP AND THINK* question:

Why does using two different enzymes to cut the plasmid prevent the plasmid from reforming a circle without the inserted gene? *Because the sequences of the*

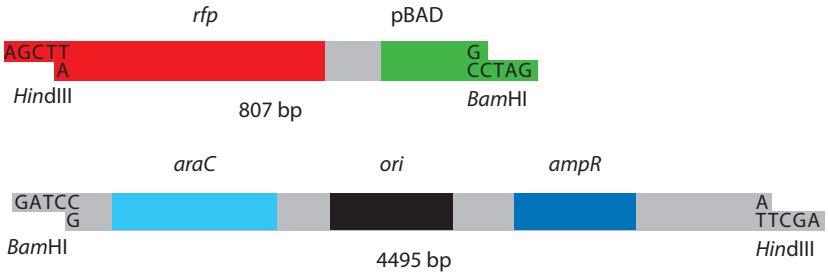
sticky ends are different (one has the Bam*HI sequence and the other the* Hind*III sequence), the two ends cannot anneal. The only way for the circle to reforming is with the insertion of the* rfp *gene that has the* Bam*HI and* Hind*III ends.*

Possible answers to the *Before the Lab* questions:

1. Review **Figure 2A.3**. If pARA-R is digested with *Bam*HI and *Hin*dIII, what fragments are produced? Record the nucleotide sequence of the sticky ends and the length of each fragment (bp), and indicate the genes and other important sequences present on each fragment.

   Sequences are as follows:

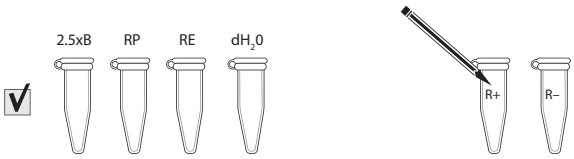
   pARA-R digestion fragments:



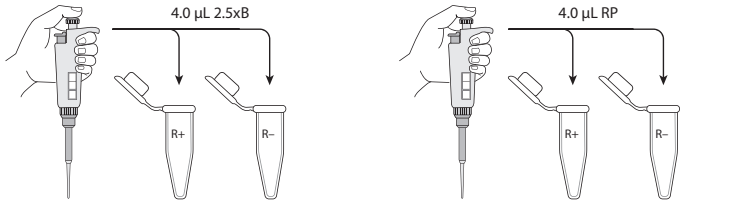
2. In order to create a plasmid that can produce RFP in bacteria, what components are needed in the plasmid? *The plasmid needs an origin of replication, a promoter, the gene of interest (*rfp*), and a gene for antibiotic resistance that allows for identification of bacteria that have taken in the plasmid.*

3. Bacteria can be killed by an antibiotic unless they carry a plasmid that has the gene for resistance to that antibiotic. Scientists call these kinds of genes selectable markers; only bacteria that carry this gene will survive exposure to an antibiotic. If the uptake of DNA by bacteria is inefficient (as discussed in the reading), why is a selectable marker critical in cloning a gene in bacteria? *You want to know which bacterial cells have the plasmid and can make the protein you are purifying. The selectable marker will allow you to kill off the bacteria that do not have the plasmid with the gene of interest.*

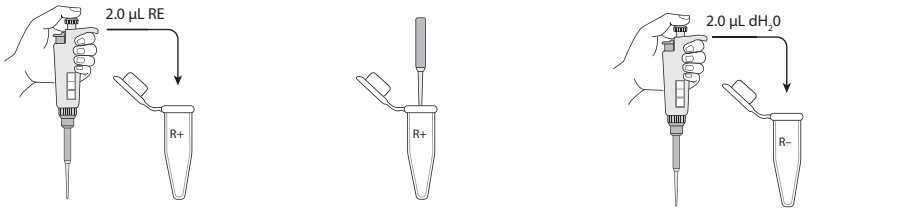
Laboratory 2A Flowchart



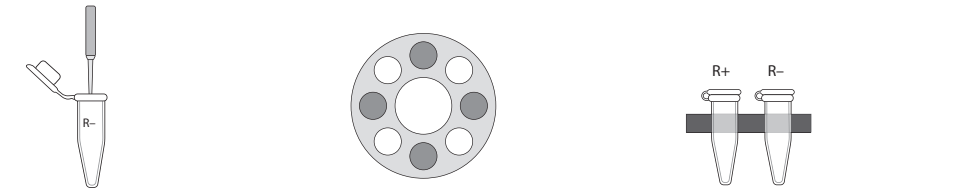
Check reagents (2.5xB, RP, RE, dH_2O) → Label 2 tubes R+ and R– → Review Table 2A.2 →



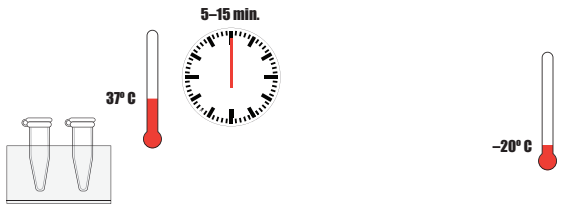
Pipette 4.0 µL 2.5xB into R+ and R– tubes → Pipette 4.0 µL RP into R+ and R– tubes →



Pipette 2.0 µL RE into R+ tube → Use pipette to mix reagents in the R+ tube → Pipette 2.0 µL dH_2O into R– tube →



Use pipette to mix reagents in the R– tube → Centrifuge the R+ and R– tubes → Place tubes in floating microfuge tube rack →



Teacher will: Place R+ and R– tubes into 37°C water bath and incubate for 5–15 minutes → Teacher will: Place the R+ and R– tubes in the freezer at –20°C

4. Read through the *Methods* section on pages 45 and 46 of the Student Guide and briefly outline the steps, using words and a flowchart. *Students' answers will vary. A student flowchart might look like the one on the following page.*

Review the *Methods* section briefly with students before they begin the lab.

LAB TECHNIQUE: Review micropipette use with students.

During the lab, students should discuss the *STOP AND THINK* questions in their groups and individually record their answers. Have students share their answers and their thinking for each question with the class.

Possible answers to the second and third *STOP AND THINK* questions:

- In this step, you are asked to set up a tube without the restriction enzymes, *Bam*HI and *Hin*dIII. What is the purpose of this step, and why is it important? *The tube without the enzymes is a control. We can compare a control with the tube that has the enzymes in order to compare the uncut plasmid with the cut plasmid. If for some reason the enzymes did not work as expected, the control will indicate that, as both the control tube and the tube with the enzymes will give the same result when examined with gel electrophoresis.*

- Why might the enzymes work best at 37°C? Why should the enzymes then be placed in the freezer? *The enzymes originally came from bacteria that are at human body temperature and are designed to work at this temperature. Placing the enzymes in the freezer will stop the reaction.*

In step 6, groups are directed to place their two microfuge tubes (R+ and R–) into the floating microfuge tube rack near the water bath. When the rack is full, place the rack in the water bath and incubate for 5–15 minutes. After the incubation is complete, place all four tubes in the freezer at –20°C for Laboratory 4A.

Have students discuss the *Chapter 2A Questions* in small groups and record their answers individually. Discuss students' answers as a class. (20 min.)

Students reflect on the concepts they learned in this chapter and show their understanding of the use of plasmids and restriction enzymes for gene cloning by answering the *Chapter 2A Questions*.

Possible answers to the *Chapter 2A Questions*:

1. List in words or indicate in a drawing the important features of a plasmid vector that are required to clone a gene. Explain the purpose of each feature. *Important features of a plasmid vector are (1) a sequence for the initiation of DNA replication,* ori, *which allows the plasmid to replicate in the*

bacteria; (2) a promoter sequence for initiating transcription of the inserted gene; and (3) a gene encoding a protein for antibiotic resistance, which allows for identification of bacteria that have taken in the plasmid.

2. What role do restriction enzymes have in nature? *They protect the bacteria from infection by bacteriophage viruses.*

3. Using your understanding of evolution, why would bacteria retain a gene that gives them resistance to antibiotics? How is the existence of bacteria with antibiotic resistance affecting medicine today? *The bacteria with the antibiotic resistance gene will reproduce more because they have a selective advantage over other bacteria that do not carry that gene. This selective advantage is a huge concern in medicine because now there are strains of bacteria that cause disease but cannot be killed by antibiotics.*

4. Bacteria, sea anemones, and humans seem, on the surface, to be very different organisms. Explain how a gene from humans or a sea anemone can be expressed in bacteria to make a product never before made in bacteria. *The DNA code and the processes of transcription and translation are the same in all living organisms. Once a human or sea anemone gene is paired with a bacterial promoter on a plasmid and the recombinant plasmid is taken up by the bacteria, the bacteria can read the DNA code and make the protein.*

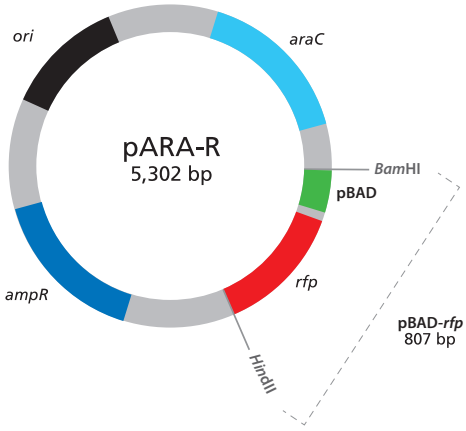
5. Due to a mishap in the lab, bacteria carrying a plasmid with an ampicillin-resistant gene and bacteria carrying a plasmid with a gene that provides resistance to another antibiotic (kanamycin) were accidentally mixed together. (Hint: Make sure that you do not kill off one of the kinds of bacteria you are trying to sort out!) *The bacteria need to be divided into two batches; one batch should be treated with kanamycin, and the other batch should be treated with ampicillin.*

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

SCIENCE BACKGROUND: THE COMPONENTS OF THE pARA-R PLASMID

Figure T2A.1: The pARA-R plasmid

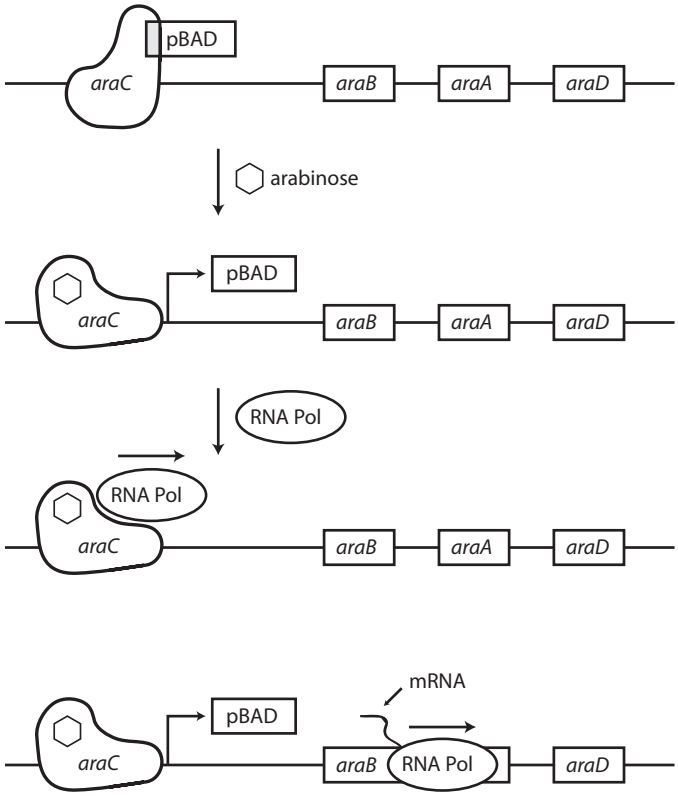


The recombinant plasmid that is used in this program to clone the *rfp* gene is the pARA-R plasmid (see **Figure T2A.1**). Many different types of plasmid vectors have been developed to clone genes. All have the basic components needed to clone and express genes in bacteria, including a sequence for initiating DNA replication (the *ori* site), a promoter for initiating transcription, a selectable marker, and a restriction site or sites near the promoter to insert the gene of interest. The pARA-R plasmid has been constructed such that the gene will be inserted using *Bam*HI and *Hin*dIII. The use of two different restriction enzymes ensures that the *rfp* gene will be inserted in only one orientation, the one that is appropriate for transcribing the sense strand of the DNA. You may want to review the idea of "sense" and "anti-sense" strands with the students.

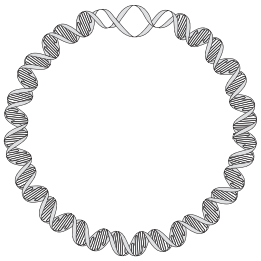
The pARA-R plasmid has been constructed to include components of the arabinose operon, which allow the expression of the *rfp* gene to be regulated. All living organisms, including bacteria, have the ability to regulate the expression of their genes. The most obvious example of this regulation is in cellular differentiation in multicellular organisms. Most cells contain the full complement of DNA, but during differentiation only certain genes are expressed as cells become skin, muscle, or root cells. Much of this regulation occurs at the level of initiation of transcription. Initiation of transcription can be turned on by proteins called activators, or turned off by proteins called repressors. While bacteria do not differentiate, they do respond to environmental conditions, such as the presence or absence of a sugar, including arabinose.

The arabinose operon is a classic example of gene regulation in bacteria (see **Figure T2A.2**). The operon comprises three genes, *araB*, *araA*, and *araD*, which code for proteins responsible for the transport and breakdown of arabinose. It also includes a promoter and a DNA sequence 5′ proximal to the promoter, which binds the AraC protein. The AraC protein regulates the expression of the arabinose genes by either allowing their transcription in the presence of arabinose or turning off gene expression in its absence.

Figure T2A.2: The arabinose operon



In the absence of arabinose, the AraC protein blocks the binding of RNA polymerase to the promoter; initiation of transcription therefore cannot occur, and the three genes—*araB*, *araA*, and *araD*—are not expressed. When arabinose is present in the environment, the sugar binds to the AraC protein, which alters the shape of the DNA in such a way that RNA polymerase can bind to the promoter and the *araB*, *araA*, and *araD* genes are expressed. The plasmid pARA-R used in this program has the pBAD promoter and the *araC* gene, as well as the genes for resistance to ampicillin and kanamycin. The *araB*, *araA*, and *araD* genes have been removed and replaced by the *rfp* gene, putting the *rfp* gene under the control of the arabinose promoter. Colonies of bacteria harboring this plasmid will be red in the presence of arabinose and white in its absence.



CHAPTER 4A

MAKING SURE YOU'VE GOT A RECOMBINANT PLASMID

OVERVIEW

Since different products are made during the ligation process, biologists must verify that they have created the recombinant plasmid they need—that is, the one with the gene of interest and all the necessary components for the protein of interest to be made. In this chapter, students learn about the importance of verifying their work as they determine if they have the pARA-R plasmid that contains both the *rfp* gene and the *ampR* gene.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- DNA is a double-stranded molecule, and each strand of DNA is made up of covalently linked subunits called nucleotides.
- A nucleotide is made up of a sugar, a phosphate group, and a nitrogenous base. There are four different nitrogenous bases—cytosine, guanine, adenine, and thymine.
- Nucleotides are attached to each other by a sugar-phosphate backbone; the nitrogenous bases jut out from this backbone.
- The two strands of DNA are connected by hydrogen bonds between adjacent nitrogenous bases, which are called base pairs; cytosine is always paired with guanine, and adenine is always paired with thymine.

LEARNING GOALS

By the end of this chapter, students will be able to do the following:

- Describe why it is important to verify products created in the genetic engineering process
- Predict the relative speed of DNA restriction fragments and plasmids through a gel during gel electrophoresis
- Separate and identify DNA restriction fragments and plasmids using gel electrophoresis

ASSESSED OUTCOMES

- Assess each student's ability to describe why it is important to verify products created in the genetic engineering process by reviewing their responses to question 1 in *Chapter 4A Questions* (page 63 of the Student Guide).
- Assess each student's ability to predict the relative distance traveled of DNA restriction fragments and plasmids through a gel by reviewing their responses to the first *STOP AND THINK* question in Laboratory 4A (page 60 of the Student Guide).

- Assess each student's ability to separate and identify DNA restriction fragments and plasmids using gel electrophoresis by reviewing their responses to questions 2–8 in *Chapter 4A Questions* (page 63 of the Student Guide).

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Review the **Introduction** and *Chapter 4A Goals* with students. (5 min.)
- Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)
- Have students read **Why Do You Need to Verify?** and answer the *CONSIDER* questions. (15 min.)
- Lead a discussion on students' answers to the *CONSIDER* questions from **Why Do You Need to Verify?** (5 min.)
- Have students begin Laboratory 4A by reading the introductory paragraph and reviewing the *Before the Lab* questions in their groups. (10 min.)

NOTE: Have students answer the *Before the Lab* questions for homework.

SESSION 2

- Have students complete Laboratory 4A. During the lab, have students share their answers to the *Before the Lab* questions and the *STOP AND THINK* questions with the class and explain their thinking. (45 min.)

SESSION 3

- Have students discuss the *Chapter 4A Questions* in small groups and record their answers individually. (20 min.)
- Lead a discussion on students' answers. (25 min.)

PREPARATION

Before you begin, you should become familiar with the laboratory procedures in this chapter, the preparation required, and the materials you'll need. The instructions assume that you will provide materials for 12 groups of 2 or 3 students. Multiply the amounts as necessary depending on the number of students and number of classes you are teaching.

COPY HANDOUTS FOR LABORATORY 4A

One copy of **DNA Ladder Diagram (RM 4A)** is needed for each student. The Reproducible Master (RM) for the handout is found at the end of this guide.

MAKE AGAROSE GELS FOR LABORATORY 4A

RESOURCES: The video, *Making an Agarose Gel* (available on the program website) goes through the process of making and casting an agarose gel as described below.

NOTE: The gels can be made several days in advance.

1. Prepare electrophoresis gel trays:

    a. Gather the following materials:

    - 6 gel electrophoresis trays
    - 12 10-well combs
    - Optional: Tape

    b. Prepare the electrophoresis gel trays for casting by securing the gates on the ends of each tray in the "up" position or taping the ends of each tray. Place a comb in each tray before adding the agarose solution.

2. Prepare the agarose solution:

    a. Gather the following materials:

    - 2 250-mL graduated flasks, one labeled "1x SB buffer"
    - 12.5 mL of 20x sodium borate buffer (20x SB buffer)
    - 237.5 mL of distilled or deionized water
    - 1.44 g of agarose
    - Mass scale
    - 500-mL flask labeled "Gel"
    - Plastic wrap
    - Disposable pipette tip
    - Microwave
    - Heat-resistant gloves or tongs

- 6 sandwich- or quart-sized resealable bags
- Waste container for used tips and microfuge tubes

b. Prepare 250 mL of 1x SB buffer by adding 12.5 mL of 20x SB buffer to one of the 250-mL graduated flasks labeled "1x SB buffer," adding distilled or deionized water to the 250-mL mark, and mixing.

c. Pour 180 mL of 1x SB buffer into the second 250-mL graduated flask.

d. Add 1.44 g of agarose (already measured in conical tubes unless otherwise noted) into the 500-mL flask labeled "Gel." Add the 180 mL of 1x SB buffer previously measured to make 0.8% agarose solution.

e. Cover the opening of the 500-mL flask with plastic wrap. Use the pipette tip to poke a small hole in the plastic wrap.

f. Place the covered flask in a microwave and heat for one minute on high. With a gloved hand, gently swirl the flask. (Alternatively, a hot plate can be used to melt the agarose, but you will need to use a double boiler.)

SAFETY: Wear heat-resistant gloves or use tongs to hold the flask.

g. Continue microwaving the flask for 5–15-second intervals until all the agarose has dissolved. To check this, hold the flask to the light and swirl the solution. Look carefully for "lenses" of agarose crystals suspended in the liquid. If none are visible, the agarose is dissolved. Wait five minutes for the agarose to cool to about 60°C before continuing to step 3.

LAB TECHNIQUE: If the solution gets too cool, the agarose begins to re-solidify. If this happens, simply reheat the solution as described above.

3. Cast the gels in the trays:

a. When the agarose solution has cooled to the point that you can safely touch the bottom of the flask (approximately 60°C; this will take around five minutes), pour 25–30 mL of the agarose solution into each electrophoresis tray. The solution should cover about 2 mm of the comb.

b. Once the gels solidify (which will take around 30 minutes), pull the comb out of each gel. Pull it straight out without wiggling it back and forth; this will minimize damage to the front wall of the well.

c. Remove the gels from the gel electrophoresis trays and store them in individual resealable bags with a small amount of 1x SB buffer. Store in the refrigerator until ready to use. Be sure to keep them flat and not on a textured surface, as textured surfaces will imprint onto the gels and impact how molecules move through them.

ALIQUOT REAGENTS FOR LABORATORY 4A

NOTE: The reagents can be aliquoted up to several days before Laboratory 4A.

1. Obtain loading dye that has been stored at room temperature. Remove the DNA ladder from the freezer and allow it to defrost for 15 minutes.

   NOTE: Loading dye is the same as the Solution 2 that was used in Laboratory 1.2; it contains orange G, bromophenol blue, and xylene cyanole.

2. Label microfuge tubes as follows:
   - 12 1.5-mL microfuge tubes marked "LD"
   - 12 1.5-mL microfuge tubes marked "M"

3. Pipette reagents into the microfuge tubes as follows:
   - 20.0 µL of loading dye into the tubes marked "LD"
   - 10.0 µL of DNA ladder into the tubes marked "M"

NOTE: After aliquoting, store the loading dye at room temperature and the DNA ladder in the refrigerator.

GATHER MATERIALS FOR LABORATORY 4A

NOTE: Gather materials on the day of the lab.

1. Prepare 300 mL of 1x SB buffer:

   a. Gather the following materials:

   LAB TECHNIQUE: You should prepare 1x SB buffer for all classes that will complete this lab—simply multiply the quantities given by the number of classes.

   - 15 mL of 20x SB buffer
   - 500-mL graduated flask labeled "1x SB buffer"
   - 285 mL of distilled or deionized water
   - 6 50-mL flasks labeled "1x SB buffer"

   b. Add 15 mL of 20x SB buffer to the 500-mL flask labeled "1x SB buffer," add distilled or deionized water to the 300-mL mark, and mix.

   c. Pour 50 mL of 1x SB buffer into each of the 50-mL flasks labeled "1x SB buffer."

2. Take the following reagents out of the freezer and allow them to defrost for 15 minutes:
   - Microfuge tube of digested pARA-R from Laboratory 2A (R+)
   - Microfuge tube of nondigested pARA-R from Laboratory 2A (R–)

3. Spin the restriction digests and controls in the microcentrifuge to pool condensation.

4. Prepare 12 sets of materials that each include the following:

    - Plastic microfuge tube rack that contains the following reagents:
        - Microfuge tube of digested pARA-R from Laboratory 2A (R+)
        - Microfuge tube of nondigested pARA-R from Laboratory 2A (R–)
        - Microfuge tube of LD (prepared above)
        - Microfuge tube of distilled water (dH_2O)
        - Microfuge tube of M (prepared above)
    - P-20 micropipette
    - Tip box of disposable pipette tips
    - Waste container for used tips and microfuge tubes (you may only need one container for every two groups)
    - Copies of **DNA Ladder Diagram (RM 4A)**, one for each student

5. Set up six electrophoresis boxes, each near a power supply; two groups will share one box. Load each box with 0.8% agarose gel (prepared above) and set one 50-mL flask containing 1x SB buffer (also prepared above) near each box. Keep the resealable bags that held the gels, and label them with each group's number and class period in case you need to store the gels before the final staining and photodocumenting is carried out—see *Completing the Gels for Laboratory 4A* below.

6. Put the microcentrifuge in a central location so that all groups can share it.

COMPLETING THE GELS FOR LABORATORY 4A

1. Unless you have a double period for class sessions 2 and 3, you will need to continue running the gels after Session 2 has ended, or interrupt the procedure if you are conducting another class using the gel electrophoresis units:

    - If you can complete the gels after the class has ended, run the gels until the orange G (yellow dye) is **near** the end of the gel. The smallest fragment you are interested in, containing the *rfp* gene, runs just behind the yellow band. Once the electrophoresis is complete, the gels can be transferred to the labeled resealable bags or to a staining tray.
    - If you need to interrupt the gels, be sure that students have been running them for at least 10 minutes. Ask students to shut off the power to the electrophoresis unit, remove the casting tray, and slide the gel into the labeled resealable bag. Place a new gel into the tray for the next class. When you have time, you can return the partially run gels to the tray and continue the electrophoresis, following the instruction above.

2. After the gels have been run, you need to stain and photodocument them to visualize the DNA bands. Instructions for staining and photodocumenting gels are included with your materials. Gels can be discarded in the regular trash following documentation.

TEACHING

SESSION 1

KEY IDEAS: In general, it is important to verify that a procedure worked as you expected it to. In biotechnology in particular, the multistep process that is used to clone a gene results in multiple products, and it is necessary to verify that you have the recombinant plasmid you need.

Review the **Introduction** and *Chapter 4A Goals* with students. (5 min.)

The **Introduction** explains the main purpose of this chapter, linking it to the Program Introduction. The *Chapter 4A Goals* tell students what they should focus on learning as they work through this chapter. Explain to students what you will assess in this chapter and what your expectations are for students' performance.

Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)

Answering the questions in this section activates students' knowledge of gel electrophoresis and verification in the lab, and reveals the gaps in their knowledge. Have students answer the questions in pairs, record their responses, and share their ideas with the class so that you can assess what they know and don't know.

Possible answers to the *What Do You Already Know?* questions:

1. Why do DNA restriction fragments and plasmids separate when analyzed by gel electrophoresis? *DNA molecules, including fragments and plasmids, move through the gel during the procedure of gel electrophoresis. Separation occurs during the procedure because lighter and more compact molecules move faster than heavier and less compact molecules.*

2. Why is it important to identify and verify a recombinant plasmid? *You might make errors during a procedure or there might be other problems, such as reagents that are not correct. The products of genetic engineering procedures are not visible, so mistakes may go unnoticed. You should ensure that you have a recombinant plasmid that has the gene you need as well as any other important genes or sequences.*

Have students read **Why Do You Need to Verify?** and answer the *CONSIDER* questions. (15 min.)

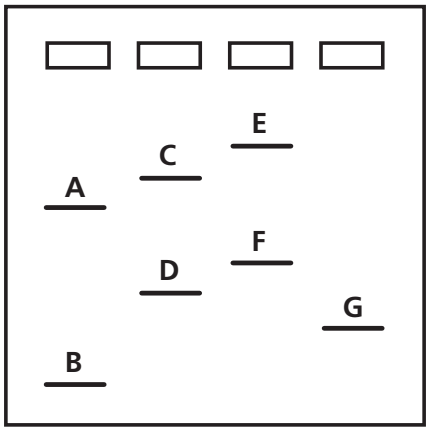
In this reading, students learn about a method for carrying out the genetic engineering process to create recombinant plasmids and to then verify the recombinant plasmid. Students also learn that plasmids can exist in three configurations: supercoiled, nicked circle, and multimer. Remind students to use the ***Glossary*** to look up scientific terms if they need help understanding the reading.

Lead a discussion on students' answers to the *CONSIDER* questions from **Why Do You Need to Verify?** (5 min.)

Assess students' knowledge of gel electrophoresis and verification in the genetic engineering process by reviewing their answers to the *CONSIDER* questions.

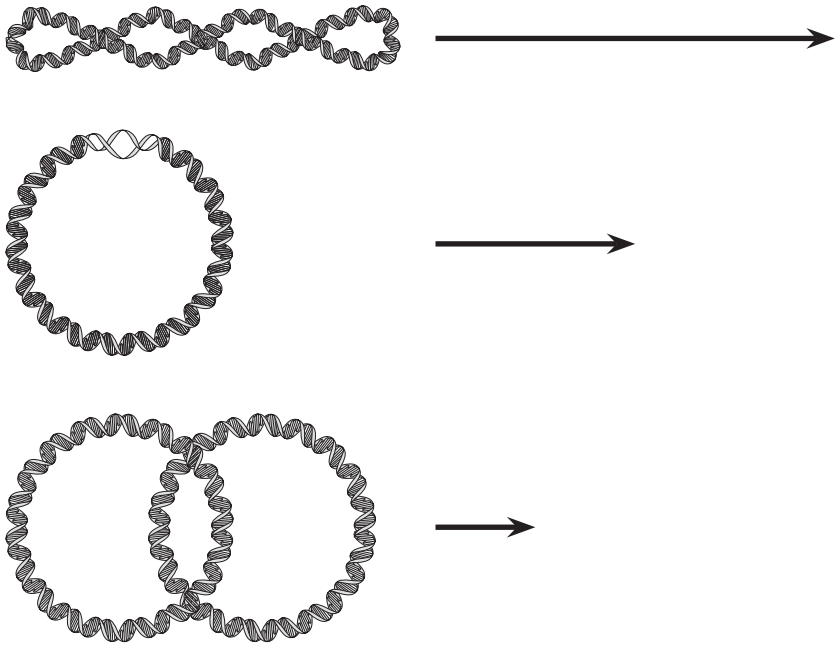
Possible answers to the *CONSIDER* questions:

- After different DNA fragments and plasmids have been separated by gel electrophoresis, the gel is stained to show bands that indicate the location of each kind of fragment and plasmid. The drawing of a stained gel below shows a series of bands that have been labeled with letters. The locations of the wells are also shown. What is the order of the fragments, from smallest to largest?



  The order of fragments from smallest to largest is B, G, D, F, A, C, and E.

- If you used gel electrophoresis to separate the same plasmid that has all three configurations, which plasmid would move the fastest, and which would move the slowest? Why do the different plasmid configurations move the way they do through the gel? Explain in words or a drawing.
  The supercoiled plasmid would move the fastest, and the multimer would move the slowest. The supercoiled and nicked circle plasmids have the same molecular weight, but the supercoiled plasmid moves more quickly through the gel because it takes up less space for its size. The multimer is very slow because it has multiple copies of the plasmid and therefore has a much greater molecular weight. See the diagram on the following page.



Have students begin Laboratory 4A by reading the introductory paragraph and reviewing the *Before the Lab* questions in their groups. (10 min.)

NOTE: Have students answer the *Before the Lab* questions for homework.

Tell students that the purpose of this lab is to verify the products of their previous lab work. Have students discuss the *Before the Lab* questions in their groups and then individually record their answers for homework. Students will need their notebooks to refer to their prior work.

SESSION 2

KEY IDEAS: DNA fragments and plasmids can be separated by gel electrophoresis. DNA cannot be seen on the gel, so a mixture of dyes, called loading dye, is mixed with the DNA samples to monitor the progress of the gel electrophoresis procedure. To help determine the sizes of unknown pieces of DNA, a mixture of DNA fragments of known sizes, called a DNA ladder, is run on the gel. After the gel electrophoresis is complete, the gel is stained in order to show the location of the DNA fragments and plasmids.

Have students complete Laboratory 4A. During the lab, have students share their answers to the *Before the Lab* questions and the *STOP AND THINK* questions with the class and explain their thinking. (45 min.)

Before beginning the lab, have students share their answers to the *Before the Lab* questions with their groups and resolve any differences. Then have students share their answers and their thinking for each question with the class.

Possible answers to the *Before the Lab* questions:

1. The pARA-R plasmid you digested in Laboratory 2A was replicated in a bacterial cell. What configurations—supercoiled, nicked circle, and multimer—might the plasmid have before digestion? *The plasmid can have all three configurations.*

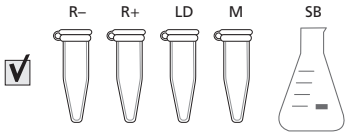
2. You need to predict all the products you might see, including the different plasmid configurations. Review your work in Laboratory 2A. What products might you expect to see in the R– and R+ tubes? Create a table that shows all the possible fragments and plasmids by tube. Include the length (bp size) of each possible fragment or plasmid, and arrange the products found in each microfuge tube by size, from smallest to largest. Include any possible plasmid configurations, and arrange them first by size and next by speed through the gel, from fastest to slowest.

   A sample table follows:

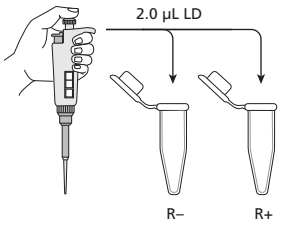
Tube	Fragments and plasmids listed in order of increasing bp size in each tube
R–	*(1) pARA-R, 5,302 bp* *The plasmid can have all three configurations, and the supercoiled configuration should move the fastest.*
R+	*(1) pBAD*-rfp *fragment, 807 bp* *(2)* ampR-ori-araC *fragment, 4,495 bp*

3. Read through the *Methods* section on pages 60 through 62 of the Student Guide and briefly outline the steps, using words and a flowchart. *Students' answers will vary. A student flowchart might look like the one on the following page.*

Laboratory 4A Flowchart

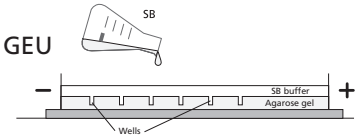


Check reagents
(R–, R+, LD, M in rack, 1x SB flask)



Pipette 2.0 µL LD into
the R– and R+ tubes

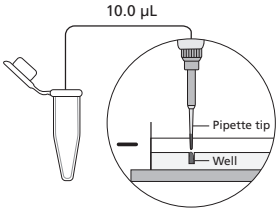
Centrifuge
the two tubes



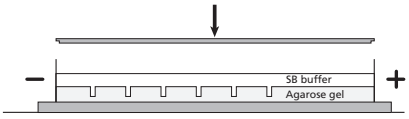
Review gel
electrophoresis
unit (GEU)

Cover gel in
GEU with 1x SB

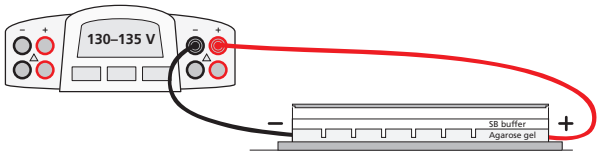
Draw location of
each well, and label
which solution is loaded
in each in notebook



Pipette 10.0 µL each of
DNA ladder (M), R–, and R+
into designated wells in GEU

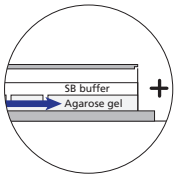


Place cover
on GEU



Connect leads
to power supply

Turn on power
supply and set voltage
to 130–135 V



Check to see if the purple
loading dye is moving toward
the positive (red) electrode

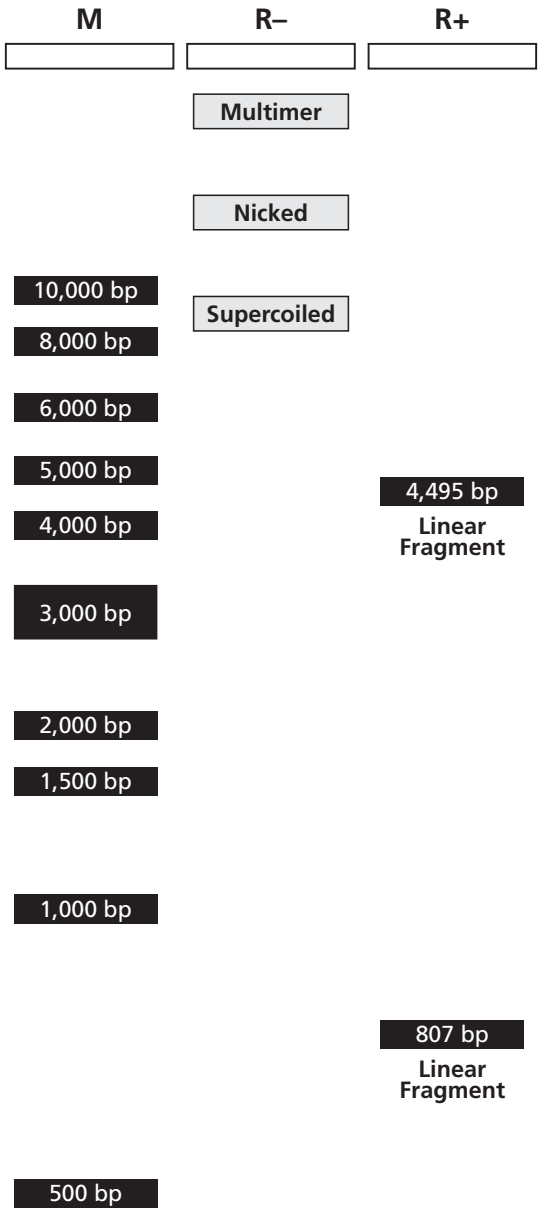
Teacher will explain
final steps

Give each student a copy of **DNA Ladder Diagram (RM 4A)**. During the lab, students should discuss the *STOP AND THINK* question in their groups and individually record their answers. Have students share their answers and their thinking for each question with the class.

Possible answers to the *STOP AND THINK* questions:

- The DNA is not visible as it moves through the gel. The loading dye contains the three dyes that you separated in Laboratory 1.2. Why is it useful to use the loading dye in this lab? *You can ensure that the samples in the gel are running and that they are running in the right direction.*

- The DNA ladder serves as a standard because it contains a mixture of DNA molecules of known sizes. By running your samples and the DNA ladder side by side in your gel, you can estimate the actual size in base pairs of unknown molecules. The **DNA Ladder Diagram (RM 4A)** shows 10 DNA bands of known sizes. Using this information, can you predict the positions of DNA bands produced by the possible products found in the K–, K+, A–, A+, and LIG tubes by indicating their position on the **DNA Ladder Diagram**? S*ee the following diagram. Note that students will not know exactly where to place the plasmids. They should know general trends, which are that: (1) plasmids are much slower than linear pieces of DNA; (2) if plasmids are the same size then difference in speed is determined by configuration with fast to slow in the order (a) supercoiled, (b) nicked circle, and (c) multimer; and (3) if plasmids have the same configuration, then the difference in speed is determined by size.*



- The DNA samples and the DNA ladder are not visible on the gel. How might the DNA be made visible once the gel electrophoresis is complete? *Unless students know about the staining methods, their answers will vary. After you have heard students' ideas, you can introduce the staining process and explain that the stain is made up of a dye that attaches to DNA molecules, just as dyes for clothing attach to fabric molecules.*

After the lab, you will need to stain and photodocument the gels for students to use to answer the *Chapter 4A Questions*. See the instructions included in the kit.

SESSION 3

KEY IDEAS: DNA fragments and plasmids that have been separated by gel electrophoresis can be identified by comparison to a DNA ladder. This identification can be used to verify if the recombinant plasmid you need is present and can also give you information about how well your procedures worked.

Have students discuss the *Chapter 4A Questions* in small groups and record their answers individually. (20 min.)

To answer many of these questions, students will need to analyze the photographs of their gels. Be prepared to help students make sense of what they see as they answer the questions.

Lead a discussion on students' answers. (25 min.)

STRATEGY: Be prepared to review students' gels with them and to talk through possible reasons for inconclusive results, such as a bad batch of plasmids, pipetting or labeling errors in the digestion procedure, inactive enzymes, and/or adding the wrong samples to the wells in the gel. Explain that scientists would check their results after each step, but students did not have enough time to carry out multiple checks.

Possible answers to the *Chapter 4A Questions*:

1. Why is it important to verify that you have the correct recombinant plasmid?
   You might make errors during a procedure, or there might be other problems, such as reagents that are not correct. The products of genetic engineering procedures are not visible, so mistakes may go unnoticed. You should ensure that you have a recombinant plasmid that has the gene you need as well as any other important genes or sequences before continuing the process of placing the plasmid in bacteria.

   STRATEGY: A similar question was also asked in the *What Do You Know?* section at the beginning of the chapter. Ask students to compare their two answers.

NOTE: Students' answers to questions 2–7 will vary, depending on their success in carrying out the procedures, including the gel electrophoresis procedure. The possible answers may not be applicable if their procedures were not successful.

GOING BEYOND: Ask students to create a standard curve for the DNA ladder fragments by plotting log bp (or kbp) versus distance on the gel on semi-log paper. Students can then use the standard curve to more accurately determine the length (number of bp) of unknown bands.

2. How did your actual gel results compare to your gel predictions? *Answers will vary, but if the procedure was done correctly, students should see the same number of bands as predicted in each lane. It is not uncommon for students to fail to add one of the necessary enzymes or to load the gels in a different order than suggested in the protocol. This is okay, as long as they can figure out what is in each lane by reviewing the undigested plasmids and the lengths of the fragments.*

3. Do you see any bands that were not expected? What could explain the origin of these unexpected bands? *Answers will vary, but unexpected bands could appear if either incorrect or expired reagents were used in the procedures, if students loaded the gels in a different order than suggested in the protocol, or if students double-loaded a lane.*

4. Does the gel show that you are using the correct recombinant plasmid? Describe the evidence you used to make this assessment. *This is the correct recombinant plasmid because the R+ lane has two fragments of the correct sizes.*

5. In the R– lane, do you see evidence of multiple configurations of plasmids? Explain your answer. *Students should see two or three different bands in the R– lane, which is evidence of multiple plasmid configurations.*

6. In the R+ lane, do you see evidence of complete digestion? Explain your answer. *Yes, there are only two bands in the lane, showing that the plasmid was completely digested into its two fragments.*

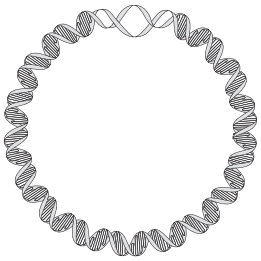
7. In which lane would you expect to find the *rfp* gene and the *ampR* gene in the gel photograph? Are you able to locate these two genes? Explain your answer. *In the R+ lane, we would expect to see a band between the 1,000 bp and 500 bp DNA ladder fragments, which is the 807 bp fragment that carries the* rfp *gene. In the R+ lane, we would expect to see a band between the 4,000 bp and 5,000 bp DNA ladder fragments, which is the 4,495 bp fragment that carries the* ampR *gene. We see both bands in these locations.*

8. Compare the lanes that have linear fragments with the lanes that have plasmids. Is there a difference in the shape of the bands between these two DNA forms? *Yes, the bands for the linear fragments have trailing edges.*

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

GOING BEYOND: As previously described, many animals have been cloned from somatic cells, but this list does not include humans. You can have students research the techniques and outcomes of cloning and the current debate on whether humans should be cloned, and then hold their own debate on this question.



CHAPTER 5A

GETTING RECOMBINANT PLASMIDS IN BACTERIA

OVERVIEW

In this chapter, students learn that recombinant plasmids need to be taken up by bacteria in order to replicate and express their genes. In the laboratory, students transform bacteria with the pARA-R plasmid that contains the *rfp* gene and the *ampR* gene.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- The relationship between DNA, genes, proteins, and traits—specifically, that genes contain the code for making a protein and that proteins are molecules that are used in making and running the cell, so they are responsible for traits
- DNA is a double-stranded molecule, and each strand of DNA is made up of covalently linked subunits called nucleotides, which are abbreviated according the nitrogenous base they contain (C, G, A, T)
- Transcription is the process by which information encoded in DNA is transferred to messenger RNA, a single-stranded ribonucleic acid
- Translation is the process by which information encoded in messenger RNA is decoded and transformed into protein

LEARNING GOALS

By the end of this chapter, students will be able to do the following:

- Describe the role of transformation in the gene cloning process
- Explain the purpose of each control in the transformation experiment
- Explain how the information encoded in a gene is expressed as a trait

ASSESSED OUTCOMES

- Assess each student's ability to describe the role of transformation in the gene cloning process by reviewing their responses to question 4 in *Chapter 5A Questions* (page 82 of the Student Guide).
- Assess each student's ability to explain the purpose of each control in the transformation experiment by reviewing their responses to questions 1 and 2 in *Before the Lab* (page 75 of the Student Guide), to the first *STOP AND THINK* question in Laboratory 5A (page 78 of the Student Guide), and to question 3 in *Chapter 5A Questions* (page 82 of the Student Guide), and their work on **Bacterial Growth Predictions (RM 5)**.
- Assess each student's ability to explain how the information encoded in a gene is expressed as a trait by reviewing their responses to questions 5 and 6 in *Chapter 5A Questions* (page 82 of the Student Guide).

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Review the **Introduction** and *Chapter 5A Goals*. (5 min.)
- Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)
- Have students read **Transforming Bacteria with Recombinant Plasmids** and answer the *CONSIDER* questions. (10 min.)
- Lead a discussion on students' answers to the *CONSIDER* questions from **Transforming Bacteria with Recombinant Plasmids**. (5 min.)
- Have students begin Laboratory 5A by reading the introductory paragraphs and answering the *Before the Lab* questions in their groups. (15 min.)

SESSION 2

- Have students continue Laboratory 5A. During the lab, have students share their answers to the *Before the Lab* and *STOP AND THINK* questions with the class and explain their thinking. (45 min.)

  NOTE: There are two 15-minute waiting periods during this lab, during which students can share their answers these questions.

SESSION 3

- Have students review the results of Laboratory 5A. (10 min.)
- Demonstrate how to set up a suspension culture for the transformed bacteria, in preparation for Chapter 6. (5 min.)
- Review transcription, translation, and the relationship among genes, proteins, and traits. (10 min.)
- Have students discuss the *Chapter 5A Questions* in small groups and record their answers individually. (10 min.)
- Lead a discussion on students' answers. (10 min.)

PREPARATION

Before you begin, you should become familiar with the laboratory procedures in this chapter, the preparation required, and the materials you'll need. The instructions assume that you will provide materials for 12 groups of 2 or 3 students. Multiply the amounts as necessary depending on the number of students and number of classes you are teaching.

LAB TECHNIQUE: The competent cells that are required in Laboratory 5A need to be stored in the freezer until the day they are used. Therefore, aliquot reagents and gather materials for the lab the same day that you carry it out. Try to minimize the number of times the cells are thawed.

COPY HANDOUTS FOR LABORATORY 5A

One copy of **Bacterial Growth Predictions (RM 5)** is needed for each student. The Reproducible Master (RM) for the handout is found at the end of the guide.

REVIEW THE SAFETY PRECAUTIONS AND WASTE DISPOSAL PROCEDURES FOR LABORATORY 5A

Review the safety precautions and procedures listed on pages 23 and 24 of this guide with students.

CALIBRATE WATER BATH FOR LABORATORY 5A

If your water bath is not already set up, see *Set Up and Calibrate Water Bath for Laboratory 2A* (page 70 of this guide). For this lab, calibrate the water bath to 42°C, making sure that there is sufficient water in the bath and that the bath is covered to reduce evaporation. Be sure to use distilled water in the water bath and keep the thermometer, timer, and floating microfuge tube rack with the water bath.

ALIQUOT REAGENTS AND GATHER MATERIALS FOR LABORATORY 5A

Gather materials on the day of the lab. After making up the racks with the reagents, be sure to store them in the refrigerator until students are ready to use them. Aliquot the competent cells 15 minutes before students begin the lab (see step 5).

1. Label microfuge tubes as follows:
    - 12 1.5-mL microfuge tubes marked "LB"
    - 12 1.5-mL microfuge tubes marked "RP"
    - 12 1.5-mL microfuge tubes marked "CC"

2. Pipette reagents into the microfuge tubes as follows:
    - 350 µL of Luria Broth into tubes marked "LB"
    - 12.0 µL of pARA-R recombinant plasmid into tubes marked "RP"

3. Prepare 12 sets of materials that each include the following:
    - Plastic microfuge tube rack that contains the following reagents (prepared above):
        - Microfuge tube of LB
        - Microfuge tube of RP
    - 2 1.5-mL microfuge tubes
    - Permanent marker
    - P-20 micropipette
    - P-200 micropipette
    - Tip box of disposable pipette tips
    - 3 Petri plates with agar:
        - 1 LB plate (one stripe)
        - 1 LB/amp plate (two stripes)
        - 1 LB/amp/ara plate (three stripes)

4. Gather the other materials needed for the lab:
    - Disposable gloves
    - Styrofoam cups (one per group)
    - Cell spreaders
    - Tape
    - Biohazard bag
    - Liquid waste collection container, such as a small beaker
    - Copies of **Bacterial Growth Predictions (RM 5)**, one for each student

5. Prepare competent cells 15 minutes before students begin the lab, as follows:
    - Prepare a small container filled with crushed ice.
    - Place CC tubes in the ice.
    - Pipette 100 µL of competent *E. coli* cells into each CC tube, holding tubes only on the rims and returning each one immediately to the ice.
    - Set up the ice-filled container of competent cells in a central location.
    - Place Styrofoam cups next to the ice container.

6. Set up the incubator in a central location, and calibrate it to 37°C.

7. Put the cell spreaders and tape in a central location so that groups can share them.

NOTE: The groups' plates will incubate for 24–36 hours at 37°C. If students do not meet in about 24 hours, remove the plates from the incubator and place them in the refrigerator. If no incubator is available, the plates may be stored at room temperature for up 48 hours.

TEACHING

SESSION 1

KEY IDEAS: Once a recombinant plasmid has been created, it must be taken up by bacteria so that it can use the bacteria cell machinery to replicate and to express the gene of interest. The process of bacteria taking up DNA from their environment is called transformation. Because bacteria are single-celled organisms that exist in a hostile environment, they are not readily transformed. However, with specific preparation, 1 in 1,000 cells will take up plasmids.

Review the **Introduction** and *Chapter 5A Goals*. (5 min.)

The **Introduction** explains the main purpose of this chapter, linking it to the Program Introduction. The *Chapter 5A Goals* tell students what they should focus on learning as they work through this chapter. Explain to students what you will assess in this chapter and what your expectations are for students' performance.

Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)

The *What Do You Already Know?* section activates students' knowledge of plasmid uptake and gene expression, and reveals gaps in that knowledge. Have students answer the questions in pairs, record their ideas, and then share them with the class so that you can assess what they know and don't know about plasmid uptake and gene expression.

Possible answers to the *What Do You Already Know?* questions:

1. Do you think that bacterial uptake of a plasmid from the environment is a common event? Why or why not? *It is probably not a common event. Cells will try to protect themselves from substances in the environment, as many of them might be damaging.*

2. What are the steps involved in transcription and translation of a gene? *During transcription, DNA is copied into messenger RNA. During translation, the messenger RNA is decoded at a cell part called the ribosome. The ribosome reads the mRNA's codons and translates it into amino acids. Each codon (a group of three bases) in the sequence corresponds to one amino acid, and amino acids are the building blocks of proteins.*

3. What is the relationship among genes, proteins, and traits (or observable characteristics)? *A gene contains the code for making a protein, and proteins are molecules that are used in making and running the cell, so they are responsible for traits. Often, a trait is the outcome of multiple proteins.*

4. What do bacteria and humans have in common that makes it possible for a human gene to be expressed in bacteria? *The DNA structure and code are the same in bacteria and humans. The cell machinery that carries out transcription and translation is the same in bacteria and humans.*

Have students read Transforming Bacteria with Recombinant Plasmids and answer the *CONSIDER* questions. (10 min.)

In this reading, students learn about transformation, which is the uptake of DNA by bacteria. The bacteria cell provides the cell machinery that allows a recombinant plasmid to replicate and express a gene. Students learn that the DNA code and the processes of transcription and translation are universal, making it possible for bacteria to express a human gene. Have students record answers to the *CONSIDER* questions in their notebooks. Remind students to use the ***Glossary*** to look up scientific terms if they need help understanding the reading.

Lead a discussion on students' answers to the *CONSIDER* questions from Transforming Bacteria with Recombinant Plasmids. (5 min.)

Assess students' knowledge of gene expression, plasmid uptake, DNA, and how ligases are used in genetic engineering by reviewing their answers to the *CONSIDER* questions.

Possible answers to the *CONSIDER* questions:

- Once a gene has been inserted into a vector, what do you think is required to make the product encoded by the inserted gene? *The vector must be brought inside a cell so that its DNA can be transcribed and translated into a protein. If the vector contains an activator for the promoter such as* araC*, a substance such as arabinose might be needed.*
- Why is it important that the cell walls of *E. coli* bacteria carefully regulate which substances can enter and exit the cell? *The cell regulates the substances that can enter and exit because some substances might affect it adversely.*

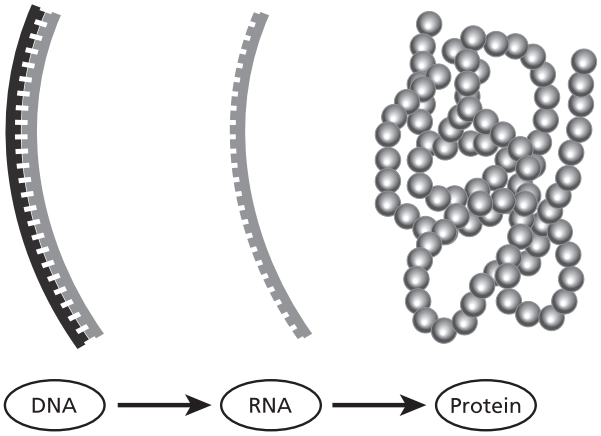
Have students begin Laboratory 5A by reading the introductory paragraphs and answering the *Before the Lab* questions in their groups. (15 min.)

Students discuss the *Before the Lab* questions in their groups and individually record their answers. This work can be completed for homework if necessary.

SCIENCE BACKGROUND: THE CENTRAL DOGMA AND REVERSE TRANSCRIPTASE

In 1957, Francis Crick laid the intellectual groundwork for understanding how information in DNA results in traits of organisms. He proposed what came to be known as the "central dogma," which states that genetic information stored in DNA is transferred to RNA in the process of transcription. The "messenger" RNA (mRNA) then carries this information to ribosomes, where it is translated into proteins (see **Figure T5A.1**).

Figure T5A.1: Central Dogma

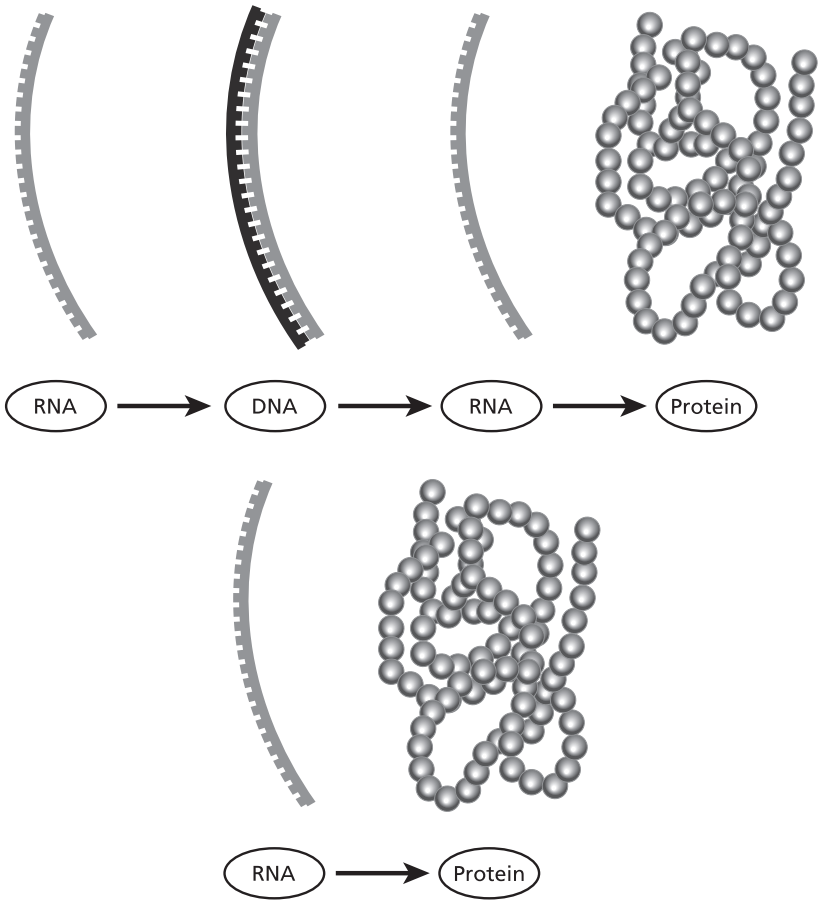


The discovery that certain viruses do not use DNA but rather RNA as the genetic material led to a significant modification of the central dogma. One group of RNA viruses, called retroviruses, store their genetic information as double-stranded RNA, and the information in the RNA is converted to DNA by the enzyme reverse transcriptase. This DNA is integrated into the host cell genome and then transcribed by the host cell RNA polymerase into mRNA, which is translated by the host cell protein synthesis machinery to generate viral proteins.

Another group of viruses, called positive strand viruses, bypass DNA completely. A single-stranded RNA molecule serves as both the genetic material and the mRNA. Polio virus is an example of a virus whose genomic RNA has three functions: (1) storing genetic information, (2) serving as a template for replication by an RNA-dependent RNA polymerase, and (3) acting as an mRNA that directs translation of the encoded information into viral proteins.

These discoveries demonstrated that the flow of information as stated in the central dogma has multiple pathways. In addition to the pathway shown above, there are two additional pathways, shown in **Figure T5A.2**.

Figure T5A.2: Alternative pathways for the Central Dogma



The discovery of reverse transcriptase, which won a Nobel Prize in 1970 for its discoverers, Howard Temin and David Baltimore, provided an invaluable tool for biotechnology. With reverse transcriptase, copies of DNA could be generated directly from mRNA. This copy DNA (cDNA) alleviated the problems presented by the fact that genomic DNA could not be used in bacteria, which do not have the ability to splice (remove) introns from mRNA. The cDNA contains only exons and therefore can be used in bacteria to produce the encoded protein.

In addition to carrying genetic information, some RNA molecules can act like enzymes and are involved in the regulation of gene expression. The ability of RNA to play a number of different roles in the cell has led some scientists to propose that the ancestral form of life was RNA-based and subsequently evolved into the forms we know now, with DNA as the major biomolecule of inheritance.

SESSION 2

KEY IDEAS: Bacterial cells that have been transformed with the pARA-R plasmid are identified by growing them in the presence of ampicillin and arabinose. Ampicillin will prevent the growth of cells that do not carry an ampicillin resistance gene, and arabinose will activate the bacteria promoter that controls expression of the *rfp* gene.

Have students continue Laboratory 5A. During the lab, have students share their answers to the *Before the Lab* and *STOP AND THINK* questions with the class and explain their thinking. (45 min.)

Students carry out a laboratory activity, Laboratory 5A, in which they transform bacteria with the pARA-R plasmid that contains the *rfp* gene and the *ampR* gene. Students learn about the importance of controls when they select for bacteria that have taken up the pARA-R plasmid.

Have students complete steps 1–6 in the *Methods* section first. Then, while the P– and P+ tubes are on ice for 15 minutes, have students share their answers to question 3 in *Before the Lab*. (All *Before the Lab* questions and possible answers follow.)

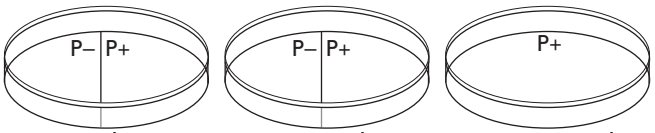
Possible answers to the *Before the Lab* questions:

1. Ampicillin is an antibiotic that kills bacterial cells by disrupting the formation of cell walls. However, the pARA-R plasmid has the ampicillin-resistance gene, which produces a protein that breaks down ampicillin. What is the purpose of growing bacteria that have been transformed in the presence of ampicillin? *Only cells that have a plasmid that contains the gene for ampicillin resistance will be able to grow in the presence of ampicillin. You can select the cells that have been transformed, which are very few.*

2. What will happen when bacterial cells that contain the pARA-R plasmid are not given arabinose? *The* rfp *gene cannot be expressed unless the cell is given arabinose—only in the presence of arabinose will the AraC activator protein turn on the* rfp *gene promoter.*

GOING BEYOND: You may want to provide more in-depth information to students about how the AraC activator protein works. The *araC* gene is part of the arabinose operon, which is a classic example of gene regulation in bacteria (see **Figure T2A.1** on page 84 of this guide). In addition to *araC*, the operon comprises three genes, *araB*, *araA*, and *araD*, which code for the proteins responsible for the transport and breakdown of arabinose. It also includes a promoter and a DNA sequence 5′ proximal to the promoter, which binds the AraC protein. The AraC protein regulates the expression of the arabinose genes by either allowing their transcription in the presence of arabinose or turning off gene expression in its absence. In the pARA-R plasmid, the arabinose operon controls the expression of the *rfp* gene, and RFP can only be made when arabinose is present.

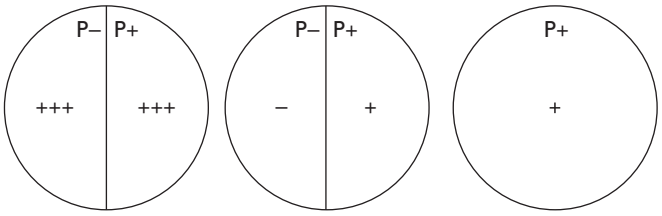
3. In the lab, you will add samples of the control group P– and the treatment group P+ to plates that contain various combinations of Luria Broth (LB), ampicillin, and the sugar arabinose. The plates will be arranged as follows:



LB plate LB/amp plate LB/amp/ara plate

Using the key on **Bacterial Growth Predictions (RM 5)**, show your predictions for the growth you would expect for each combination. Then fill in **Table 1** and **Table 2** in the handout by describing the conclusions that can be drawn if the predicted growth occurs or does not occur.

Predictions for each plate:



LB plate LB/amp plate LB/amp/ara plate

*Responses for **Table 1** and **Table 2** on **RM 5**:*

Table 1: P– Control Group (Non-Transformed Bacteria)

Plate Contains:	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)	+++	*The non-transformed bacteria are alive, and Luria Broth is able to support their growth*	*Problem with bacteria, Luria Broth, or methods*
Luria Broth ampicillin (LB/amp)	-	*Ampicillin kills the non-transformed bacteria*	*Problem with ampicillin*

Table 2: P+ Experimental Group (Transformed Bacteria)

Plate Contains:	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)	+++	*The transformed bacteria are alive, and Luria Broth is able to support their growth; bacteria were not affected by the transformation process*	*Problem with bacteria, Luria Broth, or transformation process*
Luria Broth ampicillin (LB/amp)	+	*Some transformed bacteria have plasmids with ampicillin resistance*	*Transformation did not work*
Luria Broth ampicillin arabinose (LB/amp/ara)	+	*Some transformed bacteria have plasmids with ampicillin resistance*	*Problem with arabinose; transformation did not work*

STRATEGY: Many students struggle to understand controls and will benefit from a discussion of growing the non-transformed and transformed bacteria under each condition. Help students understand that each control is designed to provide an answer to a useful question, namely:

- **Are the cells viable?**
- **Does ampicillin kill off untransformed cells?**

- **Are the cells viable after the transformation procedure?**
- **Was the transformation successful to the extent that some cells have acquired ampicillin resistance?**
- **Do any transformed cells produce RFP?**

You might ask students to consider a scenario in which cells were transformed and placed on the LB/amp/ara plate, but then did not grow—and you had not carried out any controls. Help students understand that only if the experimenter has grown the bacteria under each condition can he or she determine why the transformation procedure didn't work.

If students wonder why untransformed cells are not placed on the LB/amp/ara plate, you might walk them through thinking about whether this answers a useful question that sheds light on the success of the experiment.

4. Read through the *Methods* section on pages 77 through 81 of the Student Guide and briefly outline the steps, using words and a flowchart. *Students' answers will vary. A student flowchart might look like the flowcharts on pages 119 and 120 of this guide*.

While the P– and P+ tubes are incubating in step 11, have students discuss the *STOP AND THINK* questions and individually record their answers. Have students share their answers and their thinking for each question with the class.

Possible answers to the *STOP AND THINK* questions:

- How is the P+ bacteria culture treated differently from the P– bacteria culture? (A *culture* is an isolated population of cells.) What is the purpose of the P– bacteria culture? *The P+ bacteria culture is mixed with the recombinant plasmids, while the P– bacteria culture is not mixed with the recombinant plasmids. The purpose of the P– bacteria culture is to make sure that the cells and growing conditions work as expected. The P– bacteria culture should grow well in Luria Broth and should die when exposed to the antibiotic ampicillin.*

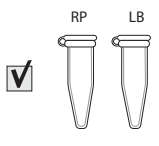
- Why do the cells need time to recover after the heat shock? *Shock implies that the heat puts the bacteria under stress. Letting the bacteria sit without subjecting them to anything else gives the cells time to return to their usual state.*

- Why are the cells incubated at 37°C? *The bacteria are adapted to growing inside the human body, which maintains that temperature.*

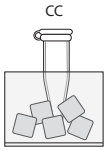
- You used aseptic technique in this lab. Why is this important? *Aseptic technique can prevent the transfer of bacteria from the experiment to the environment and from the environment to the experiment.*

At the end of the lab, remind students to place all materials that come into contact with the *E. coli* cells into the labeled biohazard bag.

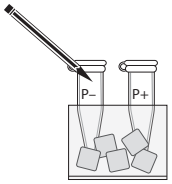
Laboratory 5A Flowchart



Check reagents (RP, LB)

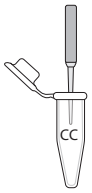


Get CC (on ice)

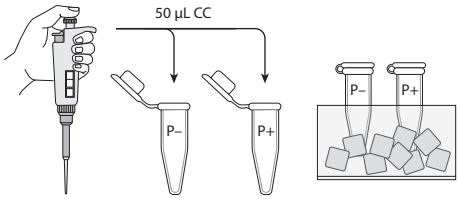


Label two tubes P– and P+ and put tubes on ice

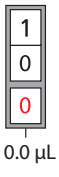
Set P-200 micropipette to 50 µL



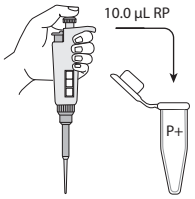
Suspend cells in CC tube by gently pumping cells in and out



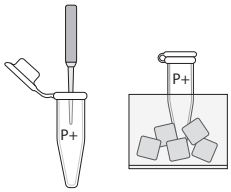
Pipette 50 µL CC into P– and P+ tubes; hold tubes at the rim to keep cold and return quickly to ice



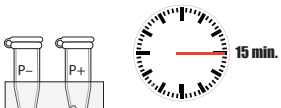
Set P-20 micropipette to 10.0 µL



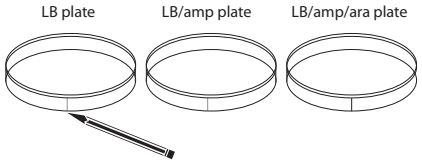
Pipette 10.0 µL RP into P+ tube, holding P+ tube at the rim to keep cold



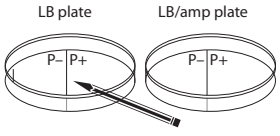
Mix reagents in P+ tube by gently pumping cells in and out; return tube quickly to ice



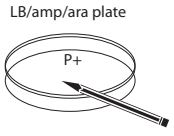
Keep P– and P+ tubes on ice for 15 min.



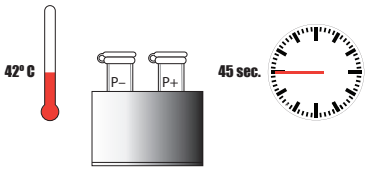
Get the three agar Petri plates (LB, LB/amp, and LB/amp/ara), and label bottom of each with group number and class period



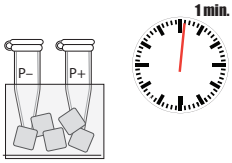
Draw a line on LB plate and LB/amp plates dividing them in middle; label one half "P–" and the other "P+"



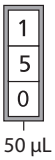
Label LB/amp/ara plate "P+"



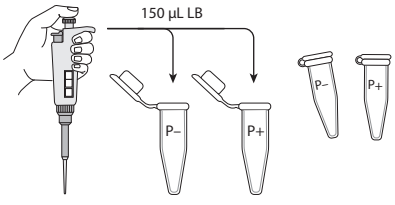
Carry cup with P– and P+ tubes on ice to the 42°C water bath, and place tubes in bath for exactly 45 sec.



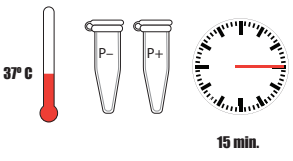
Immediately place tubes back on ice, and keep there for 1 minute



Set P-200 micropipette to 150 µL



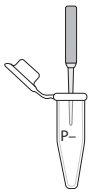
Pipette 150 µL of LB into P– and P+ tubes, cap tubes, and gently flick to mix



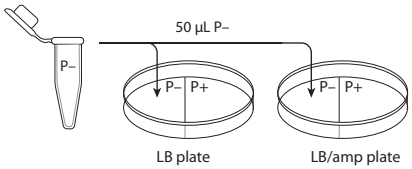
Incubate P– and P+ tubes at room temperature or at 37°C for 15 min.

Laboratory 5A Flowchart (Continued)

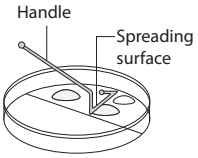
Set P-200 micropipette to 50 µL



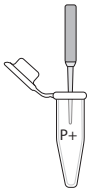
Gently pump pipette a couple of times in P– tube



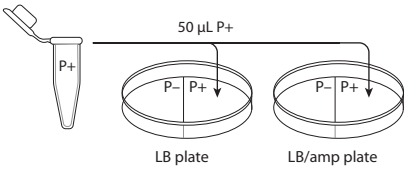
Pipette 50 µL of P– onto P– halves of LB and LB/amp plates, and close lids immediately after dispensing P–



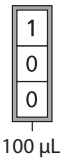
Use 1 cell spreader to spread P– cells on P– halves of LB (1st) and LB/amp (2nd) plates, and close lids



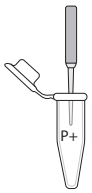
With new pipette tip, gently pump pipette a couple of times in P+ tube



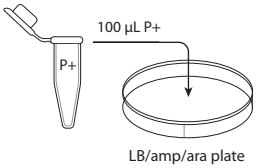
Pipette 50 µL of P+ onto P+ halves of LB and LB/amp plates, and close lid after dispensing P+



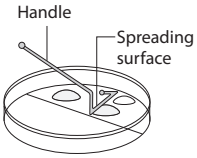
Set P-200 micropipette to 100 µL



Gently pump pipette a couple of times in P+ tube



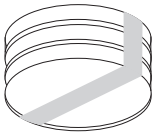
Pipette 100 µL of P+ onto various sections of LB/amp/ara plate, and close lid



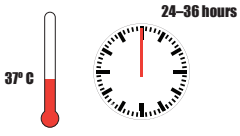
Use 1 cell spreader to spread P+ cells on P+ halves of LB (1st) and LB/amp (2nd) plates and then on LB/amp/ara plate, and close lids

Let plates sit right side up for 5 min.

Tape all three plates together, and label tape with group number and class period



Place plates in the 37°C incubator upside down



Incubate plates for 24–36 hours at 37°C or at room temperature for 48 hours

Examine plates and record the amount of growth on each

SESSION 3

KEY IDEAS: Bacterial cells transformed with the pARA-R plasmid are identified by a selection process that uses the sequences that have been engineered into the plasmid.

Have students complete Laboratory 5A. (10 min.)

Have students remove their plates from the incubators and record their results in their notebooks. If you plan to carry out Laboratory 6, save some plates that have red or bright pink colonies, and have students place the remaining plates in a biohazard bag.

Demonstrate how to set up a suspension culture for the transformed bacteria, in preparation for Chapter 6. (5 min.)

Follow the detailed instructions in the ***Preparation*** section (*Grow Bacteria for Protein Purification*, page 128 of this guide). You will remove some transformed cell colonies from plates and place them in a suspension culture in a shaker. These cells, and the pARA-R plasmids they contain, will multiply over the next few days.

Review transcription, translation, and the relationship among genes, proteins and traits. (10 min.)

STRATEGY: Reviewing important prior knowledge helps students apply what they have previously learned.

Use this review to address gaps in students' knowledge, as determined from their answers to questions 2 and 3 in *What Do You Already Know?* Refer to **Figure 5A.2** on page 71 of the Student Guide during your review.

Have students discuss the *Chapter 5A Questions* in small groups and record their answers individually. Lead a discussion on students' answers. (20 min.)

Have students reflect on their understanding of the selection process for identifying bacterial cells transformed with the pARA-R plasmid and gene expression by answering the *Chapter 5A Questions*.

Possible answers to the *Chapter 5A Questions*:

1. Look at the results of your transformation. Do your actual results match your predicted results? If not, what differences do you see, and what are some explanations for these differences? *Answers will vary. If unexpected results*

occur, help students think through what might have happened. There are many procedures in which an error might have occurred that would affect the results.

2. How many red colonies were present on your LB/amp/ara plate? *Answers will vary. All groups should get a few colonies expressing RFP, unless an error was made in carrying out the procedures.*

3. Why did the red colonies appear only on the LB/amp/ara plate and not the LB/amp plate? *The* rfp *gene cannot be expressed unless the cell is given arabinose, as the arabinose operon will only turn on the* rfp *gene promoter in the presence of arabinose.*

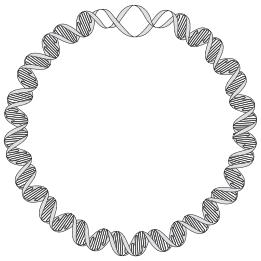
4. Recombinant plasmids are engineered so that they can replicate in the cell independently of the chromosome replication. Why is it important to have multiple copies of a recombinant plasmid within a cell? *In genetic engineering the goal is to produce a lot of product, such as insulin. More copies of the plasmid and its gene will result in the production of more product within the cell.*

5. How is the information encoded in the *rfp* gene expressed as a trait? Be sure to use what you have previously learned about gene expression and the relationship between DNA, RNA, protein, and traits. *The* rfp *gene in the plasmid is made up of DNA, and the gene is copied into messenger RNA. This process is called transcription. The messenger RNA is used to build the protein at the ribosome by attaching to transfer RNA molecules that match up codons and amino acids; the amino acids are then joined together to make the protein. This process is called translation. The protein can contribute to a trait, and in this case RFP makes the cells red.*

6. Why is it possible for bacteria to make a human protein, such as insulin, or a sea anemone protein, such as RFP? *The DNA structure and code, and the cell machinery that carries out transcription and translation, are the same across all living organisms.*

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**



CHAPTER 6

GETTING WHAT WE NEED

OVERVIEW

In this chapter, students complete the final steps in the genetic engineering process, obtaining and separating a protein of interest made by a cloned gene. Students read about bacterial cell growth, how protein conformation relates to protein function, protein folding, and the role of hydrophobic and hydrophilic amino acids in both protein folding and protein separation. In the laboratory, students lyse transformed bacteria and then use column chromatography to separate RFP from the rest of the cell proteins.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- The relationship between DNA, genes, proteins, and traits—specifically, that genes contain the code for making a protein and that proteins are molecules used in making and running the cell, so they are responsible for traits
- Bacteria undergo asexual reproduction by cell division
- Proteins are large biomolecules that consist of one or more long chains made up of building blocks called amino acids
- Proteins have many functions, including acting as an enzyme (speeding up reaction rates), transporting molecules, signaling, and forming structures
- Noncovalent bonds based on weak electrical attractions can form between different molecules or within a molecule
- Molecules or ions that are soluble in water form weak noncovalent bonds based on weak electrical attractions

LEARNING GOALS

By the end of this chapter, students will be able to do the following:

- Describe the conditions that are favorable to bacterial growth
- Explain how a protein's conformation (three-dimensional shape) is related to its function
- Explain how protein folding occurs
- Explain how column chromatography separates proteins

ASSESSED OUTCOMES

- Assess each student's ability to explain how a protein's conformation is related to its function by reviewing their responses to question 1 in *Chapter 6 Questions* (page 100 of the Student Guide).
- Assess each student's ability to describe how protein folding occurs by reviewing their responses to question 2 in *Chapter 6 Questions* (page 100 of the Student Guide).

- Assess each student's ability to explain how column chromatography separates proteins by reviewing their responses to the first *STOP AND THINK* question in Laboratory 6, Part B (page 98 of the Student Guide) and to questions 3–5 in *Chapter 6 Questions* (page 100 of the Student Guide).

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Review the **Introduction** and *Chapter 6 Goals* with students. (5 min.)
- Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)
- Have students read **Producing the Protein of Interest** and answer the *CONSIDER* questions. (20 min.)
- Lead a discussion on students' answers to the *CONSIDER* questions from **Producing the Protein of Interest**. (10 min.)

SESSION 2

- Have students read the introductory paragraph of Laboratory 6, share their answers to the *Before the Lab* questions, and complete Part A. During the lab, have students share their answer to the *STOP AND THINK* question with the class. (45 min.)

SESSION 3

- Have students complete Laboratory 6, Part B. During the lab, have students share their answers to the *STOP AND THINK* questions with the class and explain their thinking. (30 min.)
- Have students discuss the *Chapter 6 Questions* in small groups and record their answers individually. Lead a discussion on students' answers. (15 min.)

PREPARATION

Before you begin, you should become familiar with the laboratory procedures in this chapter, the preparation required, and the materials you'll need. The instructions assume that you will provide materials for 12 groups of 2 or 3 students. Multiply the amounts as necessary depending on the number of students and number of classes you are teaching.

REVIEW THE SAFETY PRECAUTIONS AND WASTE DISPOSAL PROCEDURES FOR LABORATORY 6

Review the safety precautions and waste disposal procedures from pages 23 and 24 of this guide with students.

CHROMATOGRAPHY COLUMNS

A day or two before the lab, make sure that the columns are placed upright and the stopcock is in the correct position (horizontal = locked). If the resin seems to be on the sides of any of the columns or is dry, add several milliliters of 20% ethanol to the column. Let the ethanol drain until there is about a 2-mm layer above the resin bed.

NOTE: After the chromatography lab, add 20% ethanol to the resin bed with the stopcock closed. Be sure that the columns are upright with the stopcock in the locked position.

ALIQUOT REAGENTS FOR LABORATORY 6, PART A

A day or two before the lab, make up racks with the needed reagents:

1. Label 1.5-mL microfuge tubes as follows:
   - 12 microfuge tubes marked "EB"
   - 12 microfuge tubes marked "LyB"
2. Pipette reagents into the labeled microfuge tubes as follows:
   - 200 µL of elution buffer into the tubes marked "EB"
   - 160 µL of lysis buffer into the tubes marked "LyB"
3. Cap the tubes. Store the lysis buffer in the freezer.

   NOTE: Other reagents can be stored at room temperature.

GROW BACTERIA FOR PROTEIN PURIFICATION

Exactly one day before you begin Laboratory 6, prepare a suspension culture of bacteria that have been transformed with the pARA-R plasmid (provided in your kit).

1. Gather the following materials:
    - Inoculating loop
    - Transformed cells (EC, provided in the kit)
    - Sterile flask containing LB/amp broth
    - Vented cap for flask
    - Shaker
    - Tube of sterile arabinose (500 mg/mL)
2. Prepare the suspension culture:
    - Using the inoculating loop, aseptically transfer 500 µL of transformed cells into the sterile flask containing LB/amp broth.
    - Secure the vented cap to the flask.
    - Shake and incubate the flask (at 37°C) for four to five hours. The LB/amp broth should become cloudy, indicating the cells are growing.
    - Add enough sterile arabinose to the flask so that the final concentration of arabinose is 5 mg/mL of LB/amp broth.
    - Continue to shake overnight.
    - Make sure that the culture is bright red the next morning. If it is not, the procedure was not successful.

GATHER MATERIALS FOR LABORATORY 6, PART A

NOTE: Gather these materials on the day of the lab.

1. Label 12 1.5-mL microfuge tubes "EC."
2. Pipette 1,000 µL (1 mL) of the LB/amp/ara suspension culture of *E. coli* into the tubes marked "EC."

    NOTE: Each group will need an additional 1,000 µL (1 mL) of the LB/amp/ara suspension culture of *E. coli* during the lab; one student from each group will bring you the EC tube so you can add this additional solution.
3. Prepare 12 sets of materials that each include the following:
    - Plastic microfuge tube rack that contains the following reagents (prepared above):
        - Microfuge tube of the LB/amp/ara culture of *E. coli* (EC)
        - Microfuge tube of elution buffer (EB)
        - Microfuge tube of lysis buffer (LyB)
    - Liquid waste collection container, such as a small beaker

- P-200 micropipette
- Tip box of disposable pipette tips
- Permanent marker
- Waste container for used tips and microfuge tubes (you may only need one container for every two groups)
- Biohazard bag for materials that come into contact with *E. coli* cells

4. Put the microcentrifuge in a central location so that all groups can share it.

INCUBATE CELLS WITH LYSIS BUFFER OVERNIGHT

At the end of Laboratory 6, Part A, have groups give you their EC tubes labeled with their group number and class period. Incubate the cells at room temperature overnight. If students are completing Part B the day after Part A, keep the tubes at room temperature. Otherwise, place the tubes in the freezer until the day of the lab. On the day students will complete Part B, move the frozen cell lysates to the refrigerator to thaw, except for the lysates for the first class of the day; leave those out at room temperature to thaw.

GATHER MATERIALS FOR LABORATORY 6, PART B

NOTE: Gather these materials on the day of the lab.

1. Prepare 12 sets of materials that each include the following:

    - Plastic microfuge tube rack that contains the microfuge tube of lysed cells from Part A (EC)
    - The following reagents:
        - Binding buffer (BB), 4.0 M $(NH_4)_2SO_4$
        - Wash buffer (WB), 1.3 M $(NH_4)_2SO_4$
        - Elution buffer (EB), 10 mM Tris and 1 mM EDTA[7]
        - Column equilibration buffer (CEB), 4.0 M $(NH_4)_2SO_4$

      NOTE: You will be provided with either 12 15-mL tubes of each buffer or one large container of each buffer. If you have the 15-mL tubes, one can be given to each group. If you have the larger container, pour 10 mL of each buffer into a set of flasks that can be shared by two groups.

    - 2 1.5-mL microfuge tubes
    - Chromatography column
    - Liquid waste collection container, such as a small beaker
    - P-1,000 micropipette
    - Tip box of disposable pipette tips
    - Waste container for used tips and microfuge tubes (you may only need one container for every two groups)

2. Put the microcentrifuge in a central location so that all groups can share it.

[7] EDTA is a molecular chelating agent that sequesters metal ions.

TEACHING

SESSION 1

KEY IDEAS: Transformed bacteria can be cultured and harvested to provide the protein made by the cloned gene. Separation of proteins requires an understanding of protein structure. Proteins carry out most cell functions, such as catalysis, transportation, signaling, and structure building. To carry out these functions, proteins fold into specific conformations that expose binding sites that attach to specific molecules. Separation of proteins in their folded state is difficult, so separation is achieved by unfolding proteins in salt solutions called buffers. Once unfolded, the separation method of column chromatography takes advantage of the fact that different proteins have different amounts of hydrophobic and hydrophilic amino acids. A buffer solution containing the unfolded proteins is passed through a chromatography column that has resin-coated beads, which bind the more hydrophobic proteins while the more hydrophilic proteins pass through the column. Other buffer solutions will release the bound proteins from the column by causing them to fold up again.

Review the **Introduction** and *Chapter 6 Goals* with students. (5 min.)

The **Introduction** explains the main purpose of this chapter, linking it to the Program Introduction. The *Chapter 6 Goals* tell students what they should focus on learning as they work through this chapter. Explain to students what you will assess in this chapter and what your expectations are for their performance.

Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)

The *What Do You Already Know?* section activates students' knowledge of bacterial growth and proteins, and reveals gaps in that knowledge. Have students answer the questions in pairs and record and share their ideas so you can assess what they know and don't know about bacterial growth and proteins.

Possible answers to the *What Do You Already Know?* questions:

1. How do bacteria reproduce? *Bacteria usually reproduce by binary fission, which is a kind of asexual reproduction. The cell grows and then divides to form two identical cells (sometimes called* daughter cells*) that are genetically the same as the parent cell.*

2. Why are proteins sometimes called workhorse molecules? *Proteins carry out almost all cell processes and form cell structures.*

3. How might the conformation (shape or folding) of a protein be important

for its function? Focus on one of the following protein functions: acting as an enzyme (speeding up reaction rates), transporting molecules, signaling, or forming structures. *If a protein is an enzyme (catalyst), its shape can hold reactants in an orientation that speeds up the reaction. If a protein transports other molecules or acts as a signal, its shape can match the shape of another molecule in such a way as to hold on to it. If a protein forms a structure, its shape can fit in with other proteins that also form that structure.*

4. A polypeptide is a long linear molecule when it is made, but it immediately folds into a specific three-dimensional conformation, which we call a protein. What properties of the amino acids in a protein control the folding process? *Unless students have studied protein folding previously, they may not be able to answer this question. Protein folding is the result of the formation of weak noncovalent bonds between amino acids, the tendency of hydrophobic amino acids to become buried inside the protein, and the formation of covalent disulfide bridges between sulfur-containing amino acids.*

Have students read **Producing the Protein of Interest** and answer the *CONSIDER* questions. (20 min.)

In this reading, students learn about how bacterial cells grow under favorable conditions and how protein conformation relates to its function. Students read about protein folding and learn that one factor in this process is the tendency for water-insoluble hydrophobic amino acids to be buried on the inside of proteins. Students read that proteins' relative amounts of hydrophobicity and hydrophilicity are used to separate proteins in column chromatography. Have students record answers to the *CONSIDER* questions in their notebooks. Remind students to use the ***Glossary*** to look up scientific terms if they need help understanding the reading.

Lead a discussion on students' answers to the *CONSIDER* questions from **Producing the Protein of Interest**. (10 min.)

Assess students' knowledge of how bacteria grow, protein folding and function, and how column chromatography separates proteins by reviewing their answers to the *CONSIDER* questions.

Possible answers to the *CONSIDER* questions:

- Why might the shaker flask be better at supporting bacterial cell growth than a plate? *The cells have better access to food and oxygen in the shaker because they move around freely, rather than sit on top of one another as they do on a plate.*

- If the gene of interest is controlled by an operon, such as the arabinose operon, when is the best time to turn on the gene? Keep in mind:
  - Production of the protein takes energy away from the processes of cell growth and cell division
  - A greater number of cells will produce more protein
  - Proteins can degrade over time

  Between the middle and the end of the log phase would be a good time to turn on a gene of interest. At this point there are a large number of healthy cells. Earlier in the growth curve, there are fewer cells and less protein. Later in the growth curve there are aging cells with proteins that are degrading, which may affect the ability of the cell to make protein in large amounts.

- If a mutation changes an amino acid, how might this change affect protein folding and protein function? *The change can result in an incorrectly folded protein that does not have the correct conformation to carry out its function. This mutation may cause a genetic disease.*

- If you were trying to use column chromatography to separate insulin from a mixture of proteins, would you use the same binding, wash, and elution buffers used for RFP, or would you use buffers with different salt concentrations? Explain the reasoning for your answer. *You would make new buffers with different salt concentrations to take into account the relative amounts of the proteins' hydrophobic and hydrophilic amino acids. The salt concentration of the elution buffer must cause the protein of interest to refold and be released from the column.*

SESSION 2

KEY IDEAS: To obtain a large quantity of RFP, bacterial cells that have been transformed by the pARA-R plasmid as identified by the selection process are cultured under favorable growth conditions and fed arabinose to activate the *rfp* gene. The bacterial cells are then lysed to release the cell contents.

Have students read the introductory paragraph of Laboratory 6, share their answers to the *Before the Lab* questions, and complete *Part A*. During the lab, have students share their answer to the *STOP AND THINK* question with the class. (45 min.)

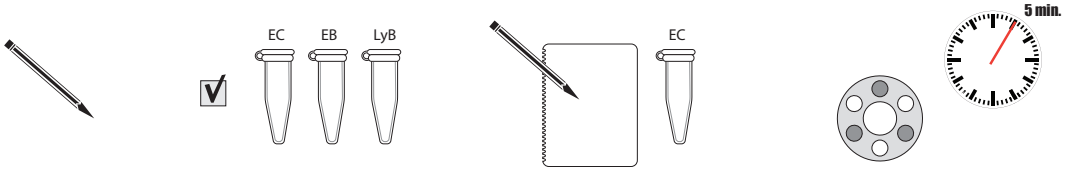
This lab takes two days. In Part A, students lyse bacteria that have been cultured and fed arabinose to activate the *rfp* gene. Students discuss the *Before the Lab* questions in their groups and individually record their answers. Have students share their answers with the class.

Possible answers to the *Before the Lab* questions:

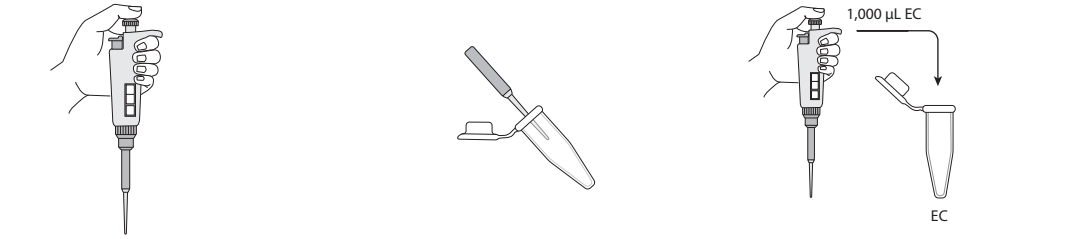
1. In column chromatography, how can solutions of different salt concentrations, which will unfold proteins to varying degrees, be used to help purify RFP? *Proteins are unfolded in the binding buffer, which has a high salt concentration. When added to the column, hydrophobic proteins in the binding buffer stick to the beads in the column, whereas hydrophilic proteins pass through the column. The hydrophobic proteins that stick to the column can be released by adding buffers with lower salt concentrations. These buffers allow the proteins to refold, which releases them from the column. Several different buffers can be added to release proteins with different relative amounts of hydrophobic amino acids.*

2. Read through the *Methods* sections for Part A (pages 96 and 97 of the Student Guide) and for Part B (on pages 98 and 99 of the Student Guide) and briefly outline the steps, using words and a flowchart. *Students' answers will vary. A student flowchart might look like the flowcharts on pages 134, 135, and 136 of this guide.*

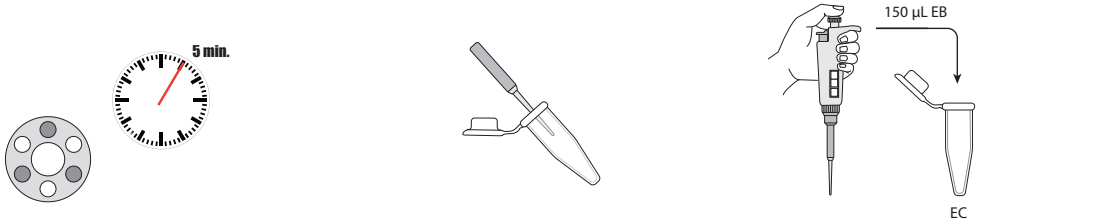
Laboratory 6, Part A Flowchart



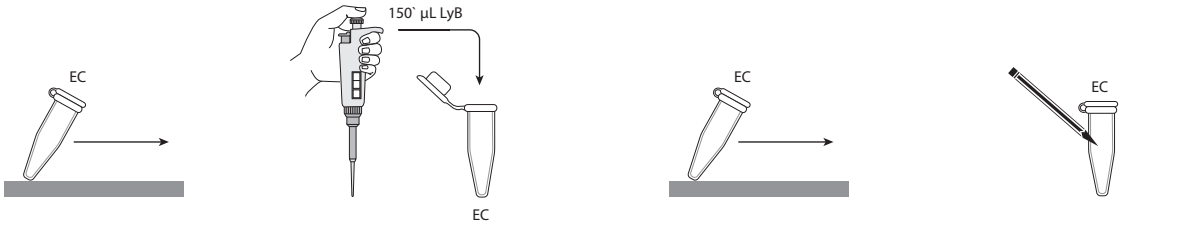
Assign tasks → Check reagents (EC, EB, LyB) → Record color of EC tube → Centrifuge EC tube for 5 minutes at max speed →



Set the P-1000 micropipette to 800 µL → Remove supernatant without disrupting pellet → Get 1,000 µL (1 mL) of additional EC solution from teacher; add to EC tube →



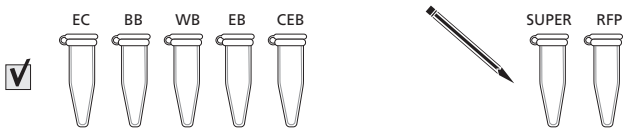
Centrifuge EC tube for 5 minutes → Use the P-1000 to remove all the remaining supernatant without disrupting pellet → Pipette 150 µL EB into EC tube →



Cap EC tube and drag vigorously across microfuge tube rack until cells are → Pipette 150 µL LyB into EC tube → Cap EC tube and drag vigorously across microfuge tube rack to mix → Label EC tube with group number and class period, and

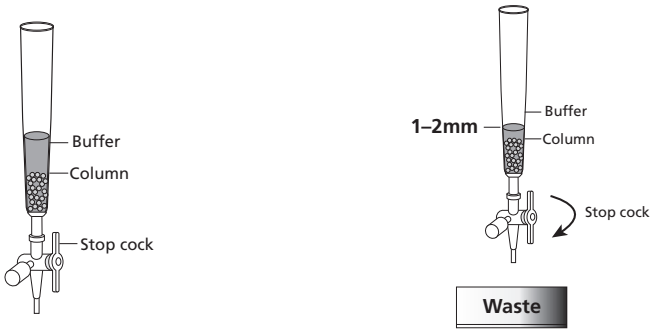
Laboratory 6, Part B Flowchart

One group member:



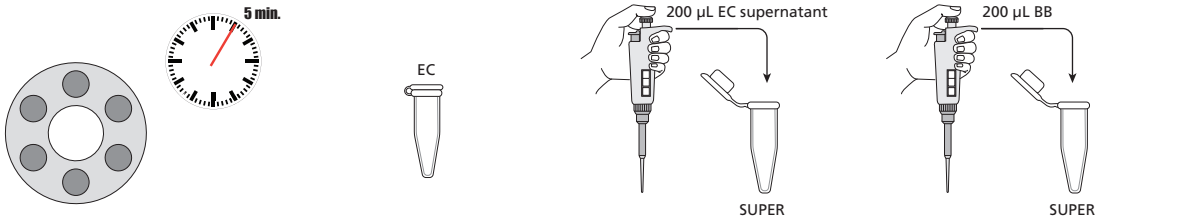
Check reagents (EC, BB, WB, EB, CEB) → Label microfuge tubes (SUPER, RFP)

One group member:



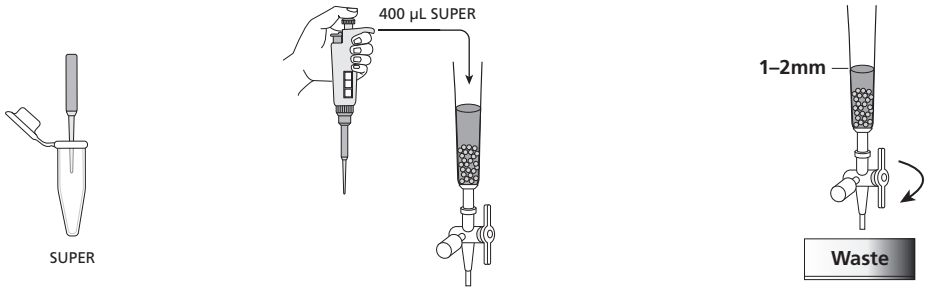
Set up chromatography column → Set waste collection container under column stopcock, open column by turning stopcock valve, allow buffer to drain from column, and close valve when 1–2 mm of liquid is left above resin

One group member:



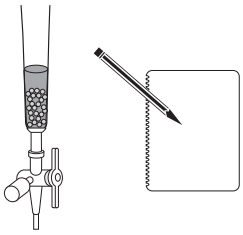
Centrifuge EC tube for 5 minutes → Examine tube → Pipette 200 µL EC supernatant into SUPER tube → Pipette 200 µL BB into SUPER tube →

Whole group:

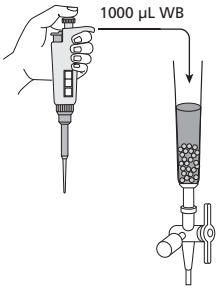


Mix reagents in SUPER tube by gently pumping solution in and out → Pipette 400 µL SUPER down side of chromatography column → Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin →

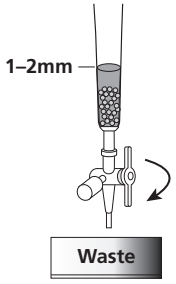
Laboratory 6, Part B Flowchart (Continued)



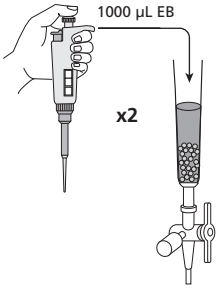
Locate RFP and record observations in notebook



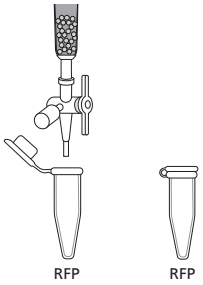
Pipette 1,000 µL WB down side of chromatography column



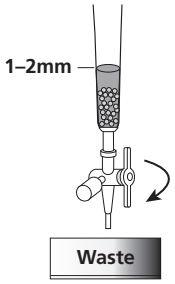
Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin



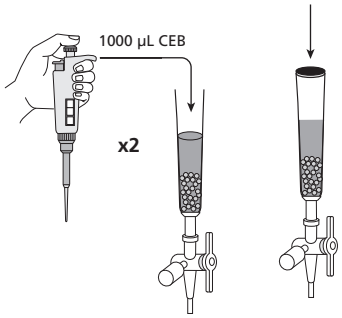
Twice more, pipette 1,000 µL EB down side of chromatography column



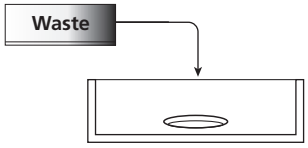
Set RFP tube under stopcock, open valve, allow only red part of eluate to drain, close valve, and cap tube tightly



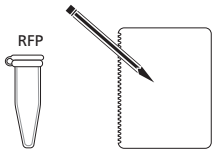
Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin



Two times, pipette 1,000 µL of CEB down side of chromatography column, and cap column tightly for next class



Pour contents of waste collection container down sink drain



Compare color intensity of RFP tube with other groups' tubes, and record observations in notebook

While the cultured cells are spinning in the microcentrifuge for five minutes in Part A, step 3, have students discuss the *STOP AND THINK* questions and individually record their answers. Have students share their answers with the class.

Possible answers to the *STOP AND THINK* questions:

- How can you determine where the RFP is in each separation step? *The RFP fluoresces red so it can be easily identified.*
- What color is the supernatant? The pellet? What are the contents of each? *The pellet is bright pink or red while the supernatant is clear. The pellet contains whole* E. coli *cells with RFP while the supernatant contains the growth medium (LB).*

RESOURCES: Help students envision protein folding by showing them a video of the process (available on the program website).

SCIENCE BACKGROUND: RECOMBINANT INSULIN

Many proteins are made up of separate peptide chains that are connected by disulfide bridges. Insulin is one such protein—it is composed of two peptide chains, referred to as the A chain (which has 21 amino acids) and the B chain (which has 30 amino acids). When making recombinant human insulin, the two chains are made separately and purified, then mixed and connected in a reaction that forms the disulfide bridges, making recombinant human insulin.

SESSION 3

KEY IDEAS: Once the bacterial cells that made RFP are lysed, the protein is separated from other proteins in the cell by using a chromatography column that takes advantage of differences in hydrophobicity. To separate RFP, which is a very hydrophobic protein, the chromatography column is packed with resin-coated beads that bind to hydrophobic proteins that have been unfolded in a binding buffer solution. A wash buffer releases moderately hydrophobic proteins from the resin and an elution buffer releases RFP from the resin. Both the wash and the elution buffers have a lower salt concentration than the binding buffer, and they cause bound proteins to refold.

Have students complete Laboratory 6, Part B. During the lab, have students share their answers to the *STOP AND THINK* questions with the class and explain their thinking. (30 min.)

In Part B of this two-day lab, students use column chromatography to separate RFP from the rest of the lysed cell proteins.

While the lysed cells are spinning in the microcentrifuge for five minutes in step 6, have students discuss the *STOP AND THINK* questions and individually record their answers. Have students share their answers with the class.

Possible answers to the *STOP AND THINK* questions:

- Three buffers you will use in this lab are the binding buffer (BB), the wash buffer (WB), and the elution buffer (EB). What is the function of each? *BB causes the proteins to unfold so that the hydrophobic proteins will bind to the resin-coated beads in the column. WB causes the moderately hydrophobic proteins to refold, and releases them from the resin. EB causes RFP to refold, and releases it from the resin.*

- What color is the supernatant? The pellet? What are the contents of each? *The pellet is white while the supernatant is pink. The pellet contains the cell debris while the supernatant contains the elution and lysis buffers (EB and LB) as well as the red fluorescent protein and other cytoplasmic proteins.*

Have students discuss the *Chapter 6 Questions* in small groups and record their answers individually. Lead a discussion on students' answers. (15 min.)

Have students reflect on their understanding of protein conformation, protein folding, and the separation of proteins with column chromatography by answering the *Chapter 6 Questions*.

Possible answers to the *Chapter 6 Questions*:

1. Why is a protein's conformation important for carrying out its function? *A specific conformation results in binding sites on the outside of the protein. The binding sites allow the protein to attach to other molecules, which is how a protein can carry out its function. Proteins have one of four functions: catalyzing reactions, transporting molecules, providing a signal, or forming structures.*

2. What properties of the amino acids in a protein relate to protein folding? *The sequence of amino acids determines folding. Protein folding is the result*

of formation of weak hydrogen bonds between amino acids, the tendency of hydrophobic amino acids to become buried inside the protein, and the formation of covalent disulfide bridges between sulfur-containing amino acids.

3. Does the eluate containing your RFP appear less bright or brighter than it did in the cell lysate following centrifugation? If there is a noticeable difference in the intensity of the red color, what might account for that? *The eluate is brighter than the cell lysate. The eluate contains mostly separated RFP, while the cell lysate contained all the cell proteins.*

4. What characteristic of RFP is used as the basis for separation by column chromatography? *RFP has more hydrophobic amino acids than hydrophilic amino acids and when unfolded sticks to the resin on beads in the column.*

5. How might the column chromatography procedure be adjusted or modified to increase the purity of the RFP sample? *Use more wash buffers that have more gradation in the concentration of salt during the elution process. Collect the red eluate in small batches and keep the middle batches only.*

STRATEGY: As you lead the discussion, use the following practices:

- **Give students time to consider one another's responses**
- **Ask for clarification**
- **Ask for an explanation**
- **Restate or rephrase**
- **Ask for an example**
- **Ask for evidence**
- **Provide examples and counterexamples**
- **Ask students to add to an explanation**
- **Ask students to evaluate a response**

RM

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LABORATORY 1.3: EXAMINING MICROPIPETTE PRECISION

You just learned that a micropipette is used to transfer very small and exact volumes of liquids in either milliliters (mL, thousandths of a liter) or microliters (µL, millionths of a liter). Each micropipette is calibrated so that the smallest volume it delivers is precise—which means that the volume measured can be consistently reproduced. In this laboratory, you will compare the precision of a micropipette and medicine dropper.

BEFORE THE LAB

Respond to the following questions with your group and be prepared to share your answers with the class.

1. Why might precision be important in the genetic engineering process?
2. Read through the *Methods* section below and briefly outline the steps, using words and a flowchart.

MATERIALS

Reagents

- A plastic microfuge tube rack with a microfuge tube of distilled water (dH_2O)

Equipment and Supplies

- P-20 micropipette (measures 2.0–20.0 µL)
- Tip box of disposable pipette tips
- 8 empty 1.5-mL microfuge tubes
- Permanent marker
- Medicine dropper
- Microcentrifuge (will be shared among all groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)

SAFETY:

- **All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.**
- **Wash your hands well with soap after completing the lab.**

METHODS

In this lab, you will examine the *precision* (exactness) of a micropipette and compare it to the precision of another instrument you've probably used a lot in your science classes: a medicine dropper.

1. Check your rack to make sure that you have the dH_2O tube.
2. Label four empty microfuge tubes "MP" and place the tubes in the rack.
3. Pipette 20.0 µL of dH_2O into each of the four MP tubes. Cap the tubes.
4. Label four empty microfuge tubes "MD" and place the tubes in the rack.
5. Use the medicine dropper to dispense a single drop of dH_2O into each of the four MD tubes. Cap the tubes.
6. Examine the water in the tubes and then centrifuge the tubes.

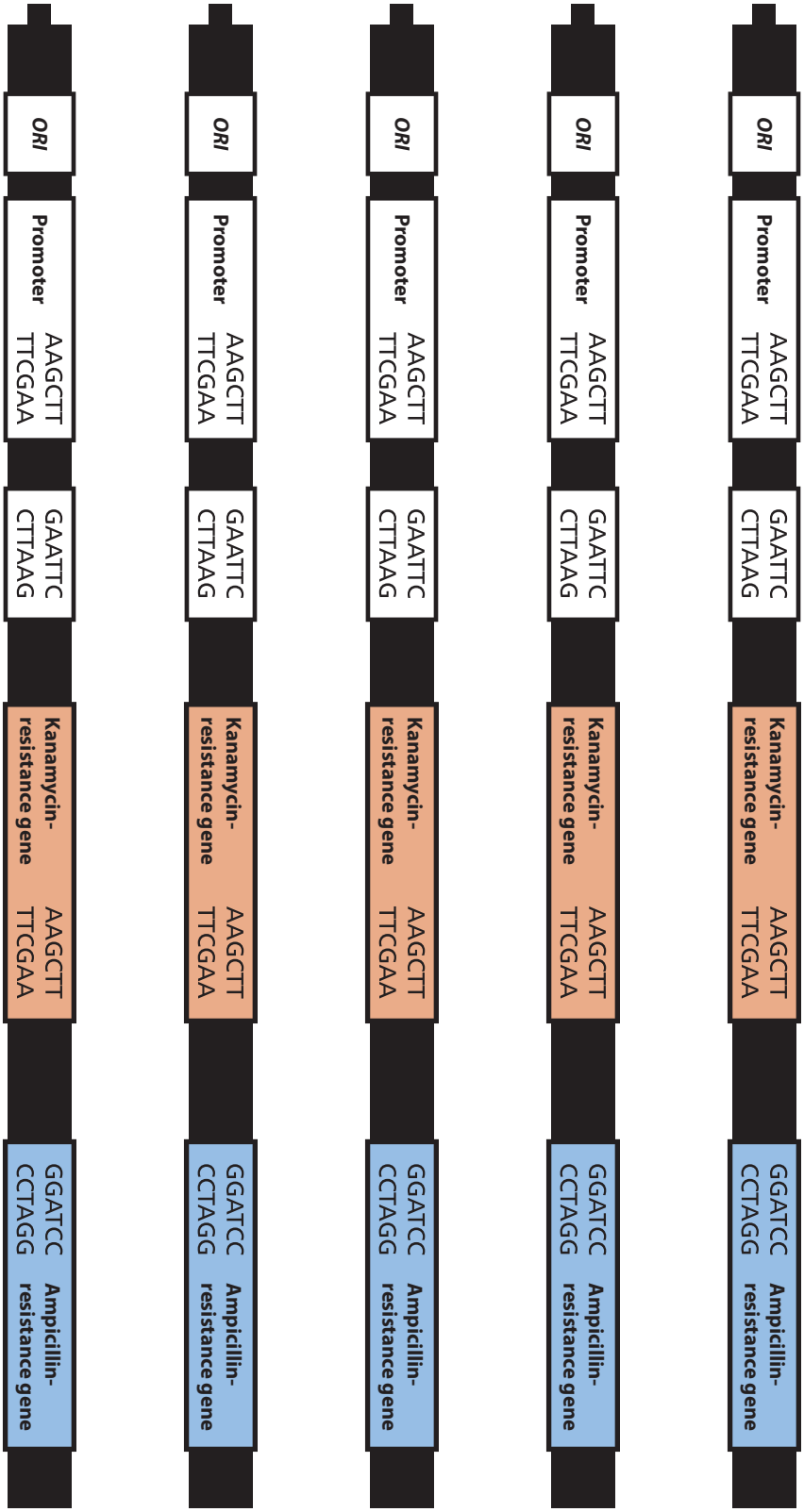
LAB TECHNIQUE: Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.

7. Re-examine the water in the tubes to verify that the water is now pooled in the bottom of the tubes.

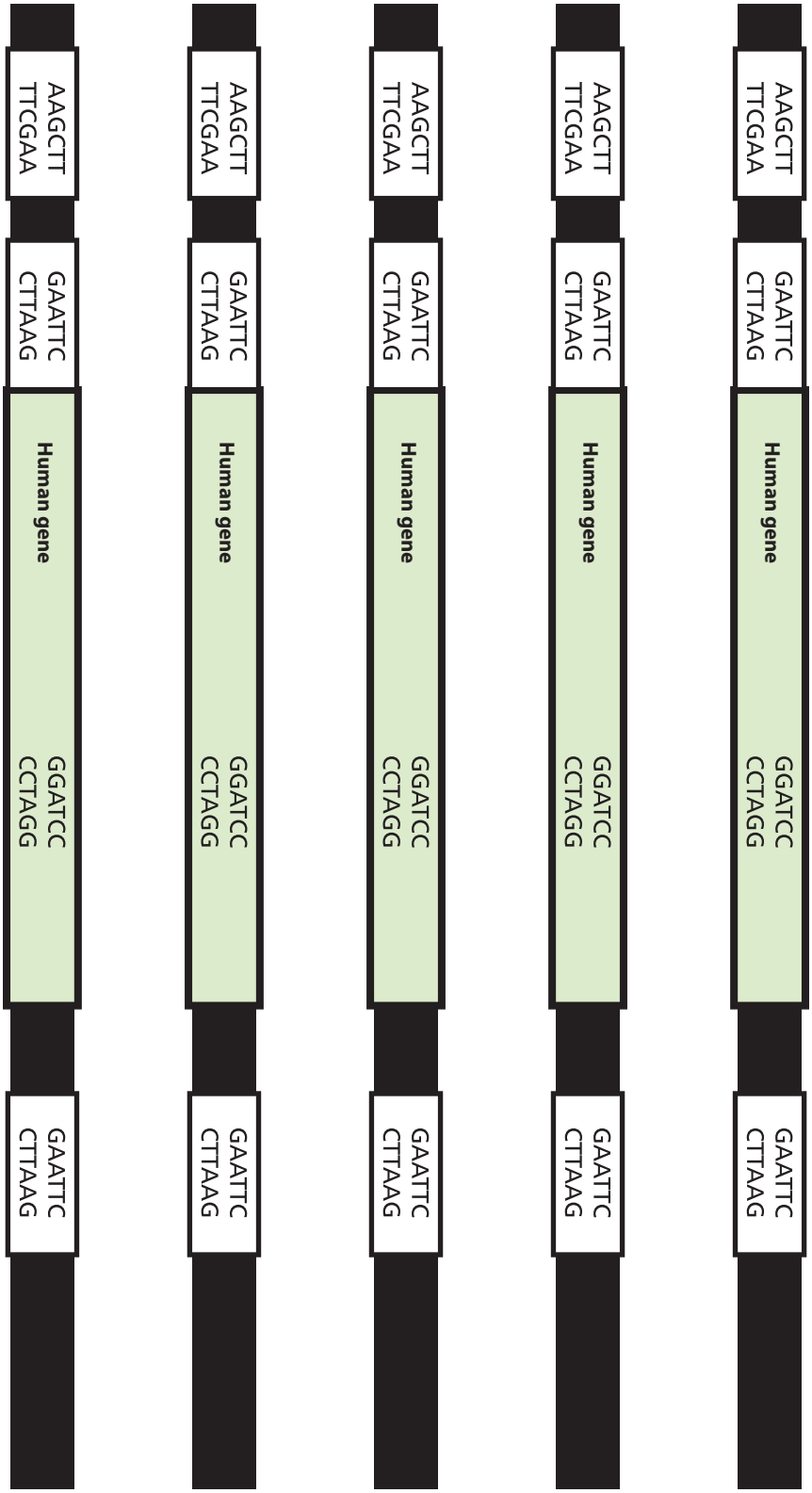
STOP AND THINK: What happens when the water is centrifuged? Why might centrifuging be important when handling small volumes of liquid?

8. Compare the amount of water in the four MP tubes. Does it appear that the same amount is in all four tubes?
9. Compare the amount of water in the four MD tubes. Does it appear that the same amount is in all four tubes?
10. Use the micropipette to check the volume in each MD tube, and compare the values you obtain.
11. Report to the class on what you learned about the amount of dH_2O dispensed by the medicine dropper. Compare the values obtained by different groups.

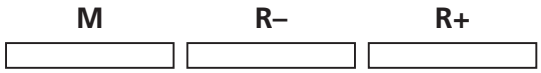
CLONE THAT GENE: PLASMID DIAGRAM



CLONE THAT GENE: HUMAN DNA SEQUENCE



LABORATORY 4A: DNA LADDER DIAGRAM



1	10,000 bp
2	8,000 bp
3	6,000 bp
4	5,000 bp
5	4,000 bp
6	3,000 bp
7	2,000 bp
8	1,500 bp
9	1,000 bp
10	500 bp

BACTERIAL GROWTH PREDICTIONS

Predict how much bacterial growth you will see on each plate. Mark the plate/plate section with +++ (for high growth), ++ (for medium growth), + (low growth), or - (for no growth):

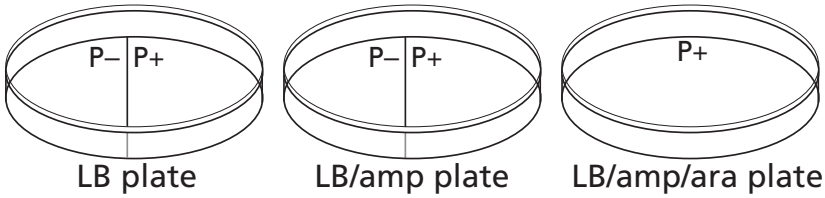


Table 1: P– Control Group (Non-Transformed Bacteria)

Plate Contains	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)			
Luria Broth ampicillin (LB/amp)			

Table 2: P+ Experimental Group (Transformed Bacteria)

Plate Contains	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)			
Luria Broth ampicillin (LB/amp)			
Luria Broth ampicillin arabinose (LB/amp/ara)			