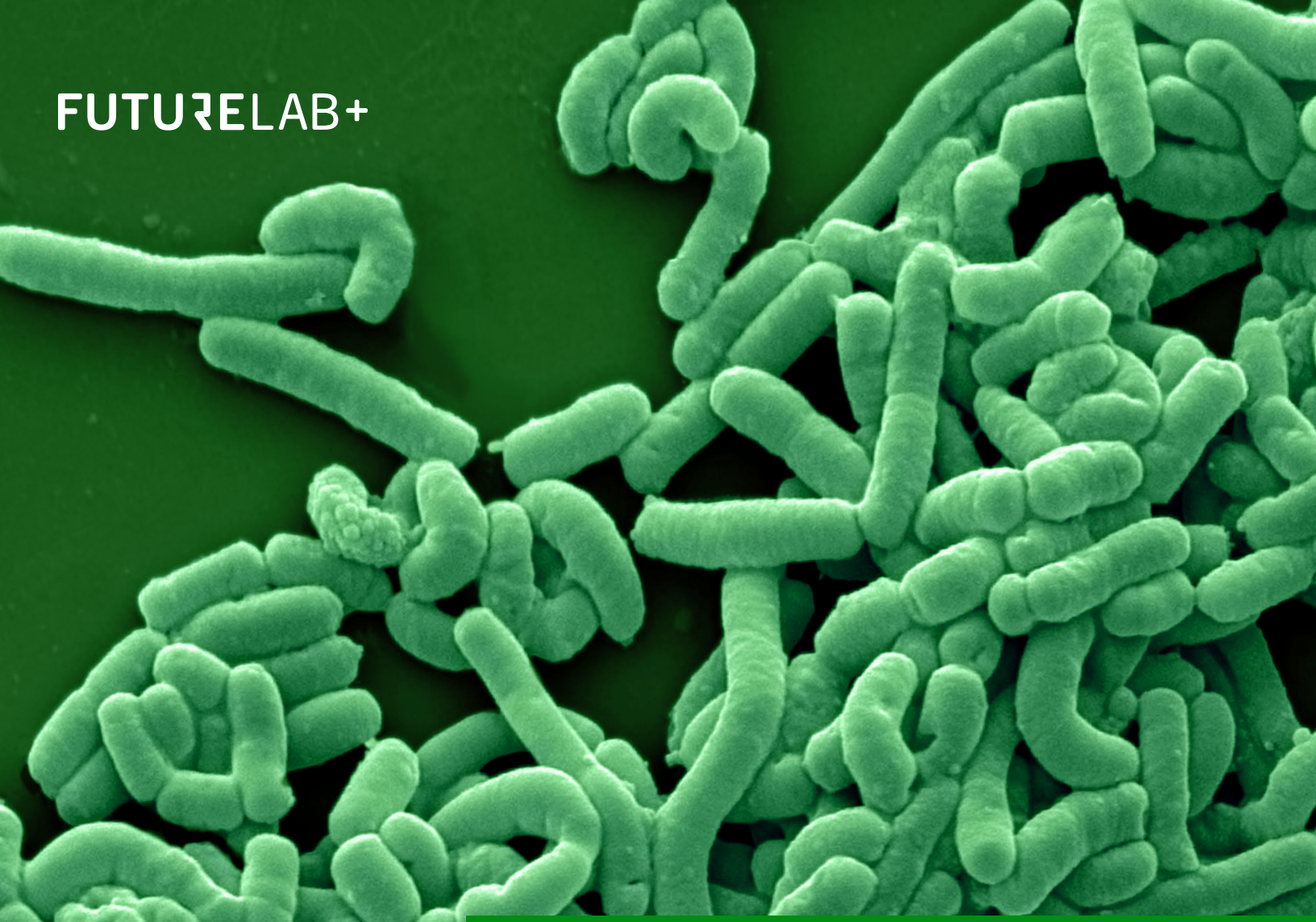
FUTURELAB+



AG/ENVIRONMENTAL

Solution Seeking Microbes

Bacterial Defense

Developed in partnership with:

Discovery Education and Ignited

In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →

This document is separated into two sections, For Teachers [T] *and* Student Resources [S]*, which can be printed independently.*

Select the appropriate printer icon above to print either section in its entirety.

Follow the tips below in the Range field of your Print panel to print single pages or page ranges:

Single Pages (use a comma): T3, T6

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Cover Image
Lactobacillus casei is one of many friendly bacteria in your gut microbiome.

AG/ENVIRONMENTAL / SOLUTION SEEKING MICROBES

Bacterial Defense

ACTIVITY DURATION

Six class sessions
(45 minutes each)

DRIVING QUESTION

How do bacteria defend themselves against pathogens?

ESSENTIAL QUESTIONS

What is the origin story of restriction enzymes and CRISPR-Cas9 in bacteria?

How do restriction enzymes work and how can we use this knowledge to benefit human health, food, or the environment?

What are the components and functions of CRISPR-Cas9?

OVERVIEW

We often think of bacteria as a threat to be feared, taking numerous precautions to avoid infections; despite this cognitive bias, however, there is much we can learn from them. In many ways, bacteria are more simplistic than human eukaryotic cells, but there are many evolutionary characteristics found among the diverse bacterial populations that can be identified and used by humans. Identifying bacterial defense systems and how they function increases the number of resources we have to utilize in combating disease, improving food production, and stabilizing the environment.

In this lesson, students will be introduced to the various ways bacteria defend against viruses, including restriction enzymes and CRISPR-Cas9. Students will initially brainstorm and observe different types of strategies for phage defense, and then focus specifically on the structure and mechanism of restriction enzymes and CRISPR-Cas9. Curiosity and observation are key characteristics of the scientists who discovered and studied CRISPR-Cas9. Also, students will be asked to create a "tweet" based on their understanding of one of the researchers. This helps students identify with the scientists.

OBJECTIVES

Students will be able to:

Describe the impact phages have on our food, global nutrient cycling, and human health.

Identify specific bacterial defenses and weapons against invading phages.

Apply the understanding of the evolutionary "arms race" between phages and bacteria to their own "superhero/supervillain."

Explain how restriction enzymes and CRISPR-Cas9 work as bacterial defense mechanisms.

BACKGROUND INFORMATION

If students have not used restriction enzymes in a previous lab, teachers can still move forward with the lesson into Day 2. However, this is also a great opportunity for a lab using restriction enzymes and gel electrophoresis if it has not already been done.

The *Restriction Enzymes* resource may be of use to the teacher, as it is a brief history of the discovery of restriction enzymes and their use in recombinant DNA. It may be helpful to recall this information before teaching the lesson.

Materials

- **Poster Paper (Drawing Models)**
- **Tape**
- **Modeling Clay**
- **Construction Paper**
- **Pipe Cleaners**
- **Toothpicks**
- **Building Blocks**
- **Popsicle Sticks**
- **Scissors**
- **Duct Tape**
- **Bacterial Defense Strategies Capture Sheet**
- **Career Profile: Kevin V. Solomon, PhD**
- **Restriction Enzymes Capture Sheet**
- **Toolkit**
- **Restriction Enzyme Capture Sheet**
- **History of CRISPR-Cas9 Reading**
- **History of CRISPR-Cas9 Capture Sheet**
- **CRISPR-Cas9 Capture Sheet**
- **CRISPR-Cas9 Interview Capture Sheet**
- **What is Sickle Cell Disease Reading**
- **What is Sickle Cell Disease Capture Sheet**
- **CRISPR-Cas9 Model**
- **mRNA Codon Chart**
- **Barriers to CRISPR-Cas9 Therapies Capture Sheet**
- **Microbe Phage Scenario Articles**

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students will analyze scenarios to predict environmental and human impacts if various bacteria that are important to an industrial or biomechanical process were to be limited by viruses (or vice versa). Students will engage in social awareness as they read about the role cultural humility plays in ensuring access to (and involvement in) the development of new therapies for sickle cell disease. Students will practice the ability to understand the perspectives of and empathize with others, including those from diverse backgrounds, cultures, and contexts.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

Students will bring real-world issues into the classroom in analyzing various impacts of bacteria and viruses on the environment in differing scenarios including health and the microbiome, food production, and ecosystems.

ADVANCING INCLUSIVE RESEARCH

In this lesson, students learn about how CRISPR-Cas9 could be used to correct single-gene mutation-caused diseases (such as sickle cell disease) in the foreseeable future, and will reflect on how it is important for the medical community to consider sources of funding and outreach programs with underserved communities that may have difficulty obtaining treatment. Students also reflect on ways the medical establishment can take community culture and beliefs into consideration when designing clinical trials for new therapies, such as CRISPR-Cas9.

COMPUTATIONAL THINKING PRACTICES

Students will focus on Pattern Recognition, seeing that both restriction enzymes and CRISPR-Cas9 use similar endonucleases to cleave foreign DNA. Additionally, the pattern of using the same Cas9, but modifying the guide RNA (gRNA), will be recognized and applied to altering the DNA of any organism. Students will also break problems into component parts, extract key information, and develop descriptive models to understand complex systems or facilitate problem-solving in analyzing the impact of relationships between bacteria or viruses and their environments and human health.

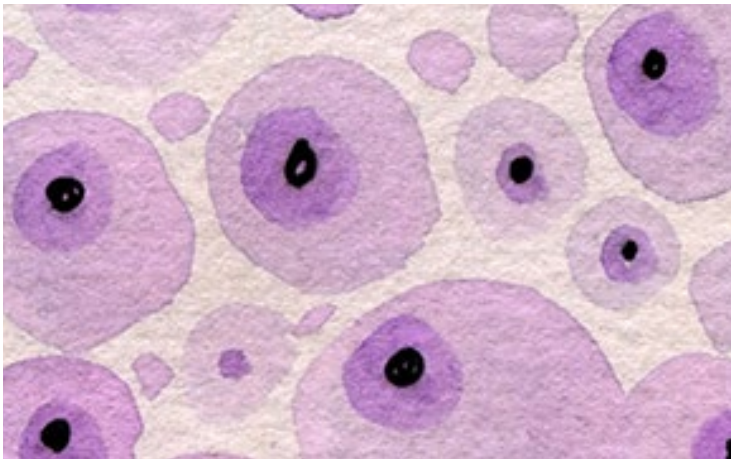
CONNECTION TO THE PRODUCT LIFE CYCLE

In this lesson, students will learn about how bacteria and our human immune systems protect themselves from invading viruses, and use this to launch research focused on how restriction enzymes and CRISPR-Cas9 have been used in gene editing. This connects to both the **discover** aspect of the product life cycle, as students learn about how and why novel products are designed, and to the **develop** aspect of the product life cycle as students learn about the technologies and methods used in creating new products.

Have you ever wondered…

How does the relationship between bacteria and phages affect the environment, human health, and food production?

Bacteria and phages are trapped in an eternal arms race, each side continually evolving new ways to fight the other and keeping each other's population in check. Although we generally think of ourselves as observers in this fight, we can be more involved and affected by the outcome. Bacteria are found pretty much everywhere on Earth, and are involved in complex interactions and relationships in the environment, human health, and food production. If these interactions are disrupted, there could be drastic consequences.



MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

As the intention is for students to identify problems and how microbes might solve them, it is important for students to first see how microbes have solved their own problems in the form of defense against viruses. Furthermore, humans have harnessed the power of microbial defense mechanisms, such as restriction enzymes and CRISPR-Cas9, and utilized them in an engineering "toolkit." Additionally, as the opening lesson involves yogurt, it is a great tie back to the initial lesson on microbes, as CRISPR-Cas9 has been in yogurt all along!

How does this connect to careers?

Molecular biologists study how changes at a molecular level impact larger cell and organism functions. They often improve lab protocols, read pre-existing research articles, and modify the genes of organisms in their research to better understand the behavior of cells.

Science writers communicate scientific research to various audiences. Some science writers translate complicated research to make it understandable to non-scientists. They might write for newspapers, magazines, or universities. Others write for a more specific audience, creating technical manuals or articles aimed at other experts in the field. Science writers use both primary literature and interviews with scientists to write their articles.

How does this connect to our world?

Viral predators of bacteria have impacts on our food, global nutrient cycles, and human health. Additionally, CRISPR-Cas9 is a new and "hot" topic, and all students should know how this connects to their own health, the environment, and the food we eat. There are ethical concerns and decisions that need to be made with CRISPR-Cas9 technology. Who makes those decisions and how these decisions are made are important for students to understand.

Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

Identify and **explain** bacterial defense strategies.

Whole Group (10 minutes)	
1	Begin the lesson by reminding students of when they made yogurt in Lesson 2. You might ask students: *If certain bacteria are growing and fermenting the milk, what might prevent them from thriving? Is there anything that they might need to protect themselves from?* Allow students to discuss in pairs. (Because students understand that phages attack bacteria and bacteria are used to make yogurt, they should make this connection fairly easily.)
2	After 3 minutes, ask students to share their ideas. They may come up with environmental factors, other bacteria, or even viruses. (If students do not make these connections, you might turn the discussion to humans and what we need to defend ourselves against: pathogens.) Remind students that bacteria have their own specific viruses, called bacteriophages, that attack only bacteria. We will explore how bacteria might protect themselves from these invaders, a part of their superhero "origin story," if you will. Teachers may also wish to tie back to Lesson 1, promoting a discussion on what a superhero may possess as a defensive tool (a hammer, spider webbing, shield, etc.). Emphasize that bacterial superheros contain their own weapons or shields to help them.

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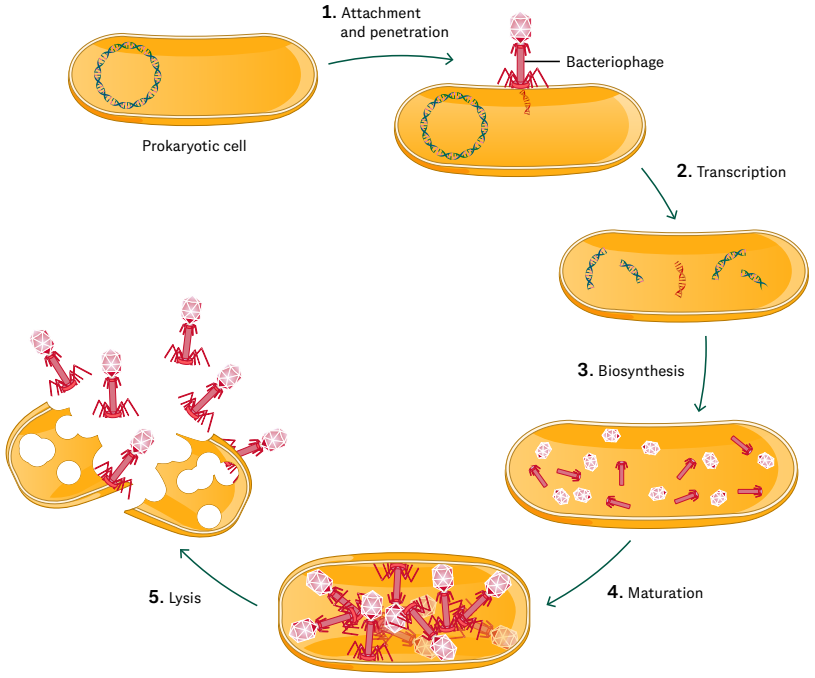
Day 1

Continued

Procedure

3 Divide students into pairs. Remind them of the phage life cycle they viewed in the previous lesson. Of course, as phages evolve to infect bacteria, bacteria will also evolve defenses. Tell students to brainstorm a way that bacteria might stop phages. Project an image like the one below, and ask students to design a defense system for a type of bacteria focusing on one phase in the phage life cycle. They should describe how to stop the virus at that phase. Using the *Bacterial Defense Strategies Capture Sheet*, they should explain how a type of bacteria could use a weapon or shield to prevent infection or future attack on other bacteria.

Lysogenic Cycle



4 Tell students to give this defensive or offensive mechanism a unique and creative name or analogy. For example, students not knowing that the actual mechanism is called preventative attachment may describe this defensive mechanism as “cloaking” to connect back to the bacterial defense (or superhero defense if connecting to Lesson 2). This is a brainstorm, and should not dive into the complexities of each defense mechanism in bacteria. The goal is for students to understand that there are many ways bacteria defend against phages.

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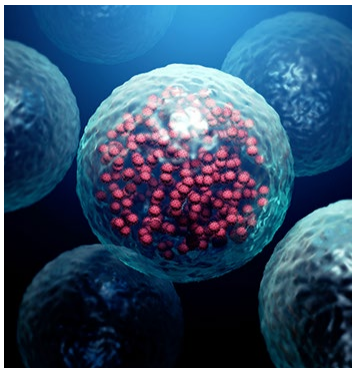
Day 1

Continued



3D illustration of a cell Infected by a virus.

Procedure

Small Group (15 minutes)	
1	Split students into six groups (you may wish to number them off 1–6). Each numbered student will research the details of a bacterial defense against phages: 1. preventative adsorption, 2. inhibiting DNA injection, 3. silencing by lysogen repressor, 4. DNA cleavage by restriction enzymes 5. toxin-antitoxin systems and abortive infection, and 6. assembly interference. You may wish to have students research together, perhaps in small groups within the larger group, as they will be explaining their defense to their classmates.
2	Provide students with materials, such as poster paper or whiteboard/ pens (you may wish to go a step further and provide 3D modeling materials if time permits, such as pipe cleaners, construction paper, tape, beads, etc.) to create a visual model of their assigned bacterial defense. Tell each student group that they will create a model of the bacterial defensive mechanism, showcasing how the mechanism works and what might happen if their bacteria did not have this defensive mechanism (for instance, without preventative adsorption, the phage DNA would be inserted into the bacteria and another defensive mechanism would have to stop the phage from replicating). Tell students they will present their models to the class.

Whole Group (15 minutes)	
1	Have student groups briefly present their models to the class, starting with Group 1. As student groups present information, tell the other groups to take notes on the unlabeled diagram of bacterial defense in their *Bacterial Defense Strategies Capture Sheet*.
2	As an Exit Ticket/Conclusion: Students will answer the first question in their **Toolkit**: *What are types of bacterial strategies for defending against phages?*

Day 2

Procedure

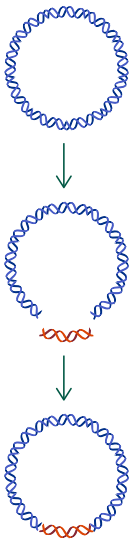
LEARNING OUTCOMES

Students will be able to:

Explain how the discovery of restriction enzymes led to recombinant DNA technology.

Describe the impact (benefits and risks) of recombinant DNA on human health, food, and the environment.

3D Illustration of CRISPR-Cas9 proteins recognizing and cutting foreign pathogenic DNA.



Whole Group (20 minutes)

1 As a whole class, review with students the six ways bacteria defend themselves against viral attack. Point out that we will focus on one specific area of defense today—the use of restriction enzymes to cut up invading viral DNA. Tell students they may recall using restriction enzymes in a previous lab activity (if they have, this is a good time to discuss how they were used).

2 Give students the *Restriction Enzymes Capture Sheet*. Tell them that today we will focus on restriction enzymes as a defense against phages in bacteria, but also on their discovery as one of the most important tools in recombinant DNA technology.

3 Play the video clip *DNALC Short: Restriction Enzymes*. As students watch, they should answer the questions in their *Restriction Enzymes Capture Sheet*. Encourage students to discuss with a partner after watching the video, then check in to see if there are questions from the whole class. Encourage students to ask questions about the discovery and use of restriction enzymes in biotechnology.

4 Tell students that if a virus or bacterium were to get into the human body, the job of the immune system is to continually attack the pathogen until it is eradicated. Show the video *How does your immune system work?* as a short review of the human immune system. Ask students: *What are similarities between the restriction enzyme bacterial defense strategy and the human immune system?* Students will most likely point out that restriction enzymes relate to the human immune system in targeting and destroying specific invaders, rendering them incapable of replicating.

Small Group (25 minutes)

1 Tell students they will now explore restriction enzymes as a tool in biotechnology. Explain that they will start by watching a short video clip of *Mechanism of Recombination* from Cold Spring Harbor Laboratory to learn how the plasmids of bacteria play a role in creating recombinant DNA.

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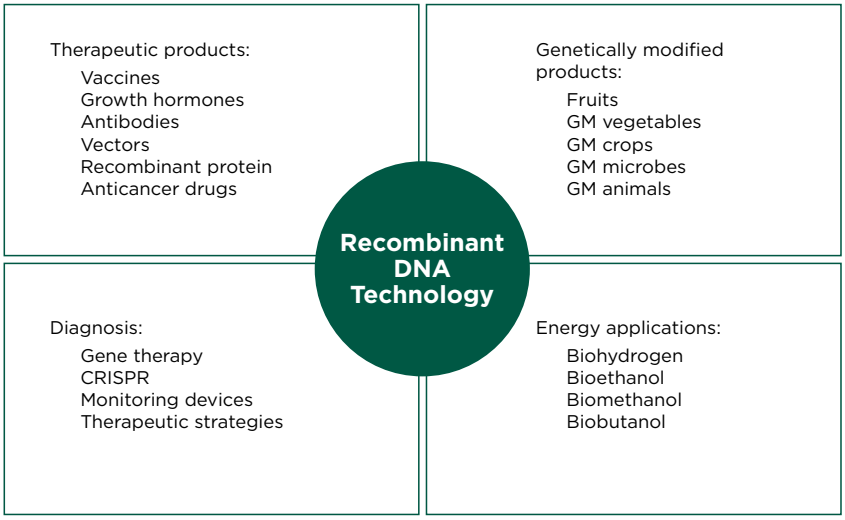
Day 2

Continued

Procedure

2 Arrange students into groups of three. Tell students to research how restriction enzymes have been applied in the following fields: health, food, and sustainability. Tell students that each member of the group is responsible for one topic and they should work together to fill in all information for each field.

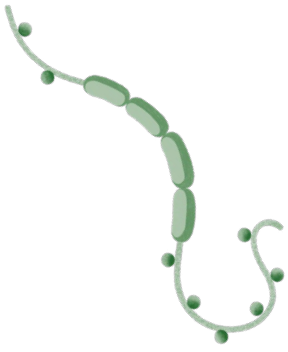
3 Share the following image with students to inspire research.



4 Tell the groups of three to assess their peers' research in order to make a Venn Diagram comparing and contrasting the three areas of study.

Individual Work/Homework

Have students add to their **Toolkit** to answer the question: *How do restriction enzymes work and how have they been used as a tool?*



Day 3

Procedure

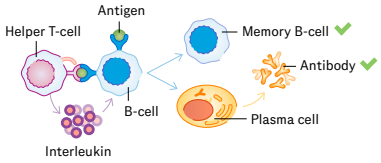
LEARNING OUTCOMES

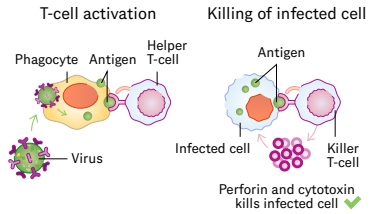
Students will be able to:

Apply the understanding of human and bacterial immunity to CRISPR-Cas9 in bacteria.

Explain how bacteria use CRISPR-Cas9 to fight off viral attacks.

B-Cells and T-Cells





Whole Group (20 minutes)

1 Ask students: *What does it mean to be immune to a pathogen?* Students may be able to answer this question, but you may need to remind them that it occurs when vaccinated, or after the immune system has already encountered the pathogen. The immune system has a memory (B-cells and T-cells) of past infections, fighting the pathogen the next time they come into contact with it without having to build up a response over a longer period of time. Symptoms often do not develop due to the immune system already having an arsenal of antibodies and white blood cells to fight the infection.

2 Tell students that bacteria can become immune too. After infection with phages, they hold the "memories" of the viruses by which they were infected. This is where the analogy ends, however, as bacteria do not have B- and T-cells. So how do they hold onto memories of past phage attacks? Tell students that they will find out by playing a game!

3 Tell students to play the *Phage Invaders: A CRISPR-Cas9 Game* to observe how bacteria defend against phages. After playing the game, tell students to discuss the following questions in pairs. Lead a class discussion, addressing any questions students had trouble understanding.

a. Q: How is the phage attacking the bacteria?

A: injecting DNA

b. Q: What does the bacteria do to defend?

A: use CRISPR-Cas9 to cut the DNA

c. Q: What happens if the bacteria do not defend against the phages?

A: They multiply and kill the bacteria.

d. Q: What happens when you hit the bacterial DNA? Why is this important for the bacteria?

A: Nothing—it is protected and will not be cut up by its own CRISPR-Cas9 system.

e. Q: Why are there different colors for the guide RNA (gRNA)? How do they behave differently?

A: They are specific to the phages and will only attack phages with which they match.

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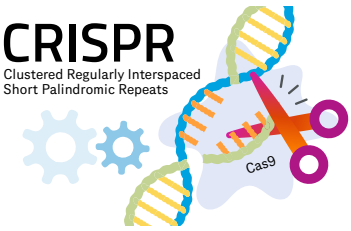
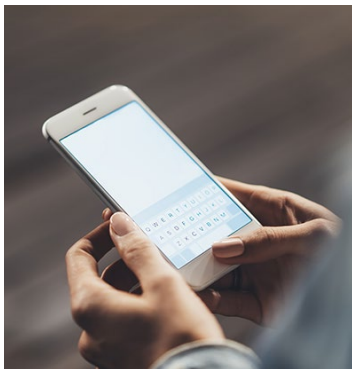
Day 3

Continued

Procedure





Small Group (25 minutes)	
1	Ask students if they have heard of CRISPR-Cas9. If so, ask what they heard about it. They might identify that it is a gene-editing tool. CRISPR-Cas9 is a bacterial defense similar to restriction enzymes. Today's lesson will focus on the scientists who contributed to the discovery and use of CRISPR-Cas9 technology.
2	Ask students to read the background on the *History of CRISPR-Cas9 Reading*, and then complete the *History of CRISPR-Cas9 Capture Sheet* to create "tweets" that one of the scientists might write on Twitter. If students are unfamiliar with Twitter, tell them to create a "phrase" that their scientist might say regarding their research with CRISPR-Cas9. The phrase must be under 280 characters long.
3	Ask students to get into groups of four and read their tweets out loud. Tell other students to guess which scientist tweeted that information.
4	Tell students to add to their **Toolkit**, answering the following essential question: *What are types of bacterial strategies for defending against phages?*

Day 4

Procedure

LEARNING OUTCOMES

Students will be able to:

Explain the steps and scientists involved in the discovery of CRISPR-Cas9.

Explain how CRISPR-Cas9 is used in biotechnology.

Identify the difference between strong and weak interview questions.

Teacher Note > *It may be necessary to begin the review in Step 1 below (with some directive instruction), reiterating the history of CRISPR-Cas9 discovery and pointing out the pieces of the CRISPR-Cas9 tool, along with what each does. One idea is to draw a representation on the board, and label each piece as you discuss the review with students.*

Small Group (15 minutes)

1	Review the *History of CRISPR-Cas9 Reading* and *Capture Sheet*, answering any questions that remain. Remind students that scientists often "stand on the shoulders of giants," and that each small step in the discovery process is key to the next step, whether it be large or small. It is upon the foundations that were laid by small discoveries that lead to huge breakthroughs in science. Tell students that they will now focus on the most influential breakthroughs in the history of DNA research since the discovery of restriction enzymes, and relate them to the tool of CRISPR-Cas9.
2	Do a web search for a "CRISPR-Cas9 mechanisms and applications interactive." In small groups, students should walk through the interactive. Students will take notes on *CRISPR-Cas9 Mechanism Capture Sheet*, explaining the components of the CRISPR-Cas9 system and the four steps of how it works (targeting, binding, cleaving, DNA repair). After students have completed the activity, review the steps by calling on student volunteers to explain the components and each step.
3	Tell students to add to their **Toolkit** to answer the essential question: *What are the components of CRISPR-Cas9 and what is the function of each?*

Whole Group (5 minutes)

Play the following three-minute video clip *2018 Kavli Prize Winners—NANOSCIENCE: Doudna, Charpentier and Siksnys* so that students can get a general sense of a few of the scientists who played a pivotal role in the discovery of CRISPR-Cas9 and its use as a tool in research.

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Day 4

Continued

Procedure

Small Group (15 minutes)

1 Place students in groups of four to role play an interview with the scientists showcased in the video. Allow students to choose which role they would like to play: interviewer, Doudna, Charpentier, or Siksnys.

2 Pass out the *CRISPR-Cas9 Interview Capture Sheet*. Each student should read their part, doing their best to portray how they think the scientists would sound responding to the questions asked.

Whole Group (10 minutes)

1 At the end of the reenactment, lead a whole class discussion on what makes for strong or weak interview questions. Review the weak questions and give an example of how to improve one of the questions. Tell students they have about five minutes to discuss with a partner how to improve the remaining weak questions. Have students discuss these improvements with the class. (Questions 3 and 7 were "weak" questions.) Tell students that strong interview questions draw out the thoughts and feelings of the person being interviewed along with details and stories. Weak interview questions are answered quickly with yes or no answers.

2 Explain to the class that in a few lessons they will be creating their own questions and conducting interviews to stress the importance and connection to the unit.

Extension Activity

Students could generate some potential interview questions for the scientists based on the background reading. Groups could then evaluate the effectiveness of those questions to get them thinking about what makes a strong interview question.

Day 5

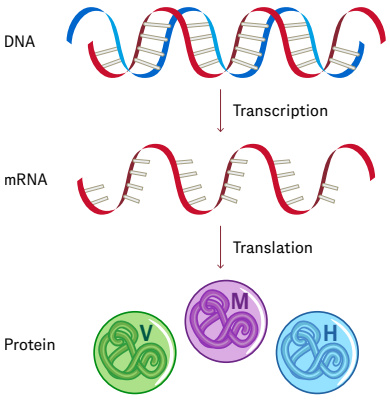
Procedure

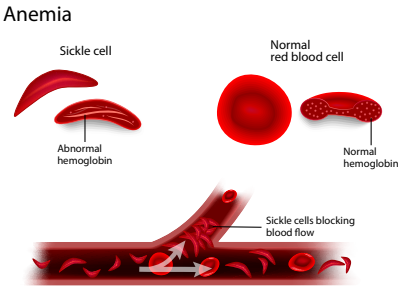
LEARNING OUTCOMES

Students will be able to:

Model the structure and mechanism of CRISPR-Cas9.

Explain how CRISPR-Cas9 can be used to treat genetic disorders.





Teacher Note > *If students are unfamiliar with protein synthesis, this would be a good time to review the central dogma (DNA to RNA to Protein).*

Small Group (40 minutes)

1	Ask students what they know about genetic diseases and their treatments. Students may respond that they have heard of cystic fibrosis, sickle cell disease, hemophilia, or progeria, and that historically, treatments have been available, but cures have not. Some students may respond that they have heard that CRISPR-Cas9 can be used to cure genetic diseases.
2	Tell students they will explore how CRISPR-Cas9 can be used to treat and maybe even cure genetic disorders. Show the clip *UCSF, Berkeley, UCLA to launch sickle cell trial using CRISPR* and have them answer the questions in the *What is Sickle Cell Disease Capture Sheet* as they watch.
3	Tell students to highlight and annotate the *What is Sickle Cell Disease Reading*. Review with students that fetal hemoglobin can be activated to help people with sickle cell disease by disabling the repressor protein that usually turns off the fetal hemoglobin gene in adults.
4	Pass out the *Using CRISPR-Cas9 to Combat Disease Capture Sheet*. Ask students to visit the National Center for Biotechnology Information (NCBI) website to view the *Crystal structure of BCL11A* repressor bound to γ-globin. Remind them that this repressor is what turns off fetal hemoglobin in adults. Explain that you will see how CRISPR-Cas9 can be used to disable the repressor in adults, turning fetal hemoglobin back "on."

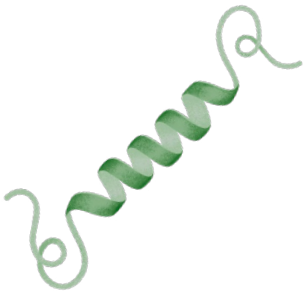
Continues next page >

Day 5

Continued

Procedure

5	Tell students to click the 3D view in the bottom left of the window to be able to move the molecules around. Point out how the protein (repressor) is represented by pink alpha helices and beta pleated sheets, while the γ-globin DNA is shown in green and gold.
6	Tell students that they will explore how CRISPR-Cas9 can be used to modify this repressor so that it cannot bind to the γ-globin gene. Before beginning, explain that the DNA and amino acid sequence will be together on one strip of paper that they will cut out and paste together. As this is a model, this is not truly indicative of how the process occurs in the cell. DNA will first be transcribed (nucleus), and then translated (cytoplasm at the ribosome). The model has been simplified so that the cut in the DNA will immediately show the change in the amino acid sequence that would be translated.
7	Have students transcribe and translate the DNA sequence on sequences A and B on their capture sheets (the sequences are the same, so they are really only doing this once). Tell them that they only need to transcribe and translate the blanks in the strip. You may wish to demonstrate the first one for them. Have students use the *mRNA Codon Chart* to translate the RNA sequence into amino acids.
8	Once the DNA is transcribed and translated, tell students to cut DNA Sequence A on the dotted lines and tape each end-to-end until they have a long chain of amino acids. Have students model protein folding by taping each numbered amino acid together using tape. For example, amino acid 1 should form a bond with amino acid 1, etc. This represents the completed, functional repressor protein. Remind students that protein structure is related to protein function. If the protein's structure were altered, the function would be lost. Tell them to place this on their desks, away from their work area to come back to momentarily.
9	Tell students they will model the use of CRISPR-Cas9 to deactivate the repressor protein. They will keep the other repressor protein in its functional state for comparison.
10	Tell students to find the PAM sequence on sequence B in the repressor so that they can design a guide RNA (gRNA) that will target the sequence. Remind them that the PAM sequence is a NGG sequence in the DNA to which Cas9 will bind.



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Day 5

Continued

Procedure

11	Following the directions in the capture sheet, students will transcribe their target sequence into a gRNA sequence. Tell them to write their target gRNA sequence on the capture sheet and to cut out the *CRISPR-Cas9 Model Capture Sheet* by making a cut on the dashed line in the middle. This is where the DNA will fit as Cas9 scans for matching target sites. **Make sure students do not cut the model in half.**
12	Tell students to use the same folding rules to match the numbers on their "string of amino acids" to illustrate a folded protein, reminding them that this is the "CRISPR'd protein." Ask students to compare the two models to determine if they look the same and discuss if they function the same.
13	Pass out the *Barriers to CRISPR-Cas9 Therapies Capture Sheet*. Remind students that knowing it can cost $10,000 per month for CRISPR-Cas9 therapy, and that *sickle cell disease (SCD) primarily affects African-Americans*, there are barriers (for example, health insurance, access to and trust in the medical establishment, among others) to access that are more complex than the expense. However, researchers are finding ways to overcome these barriers, and studies have found that cultural humility is key.
14	Show students the video, *Cultural Humility*. Ask them to discuss with their partners what they think it means to practice cultural humility, or to give an example in which they saw someone practicing cultural humility.

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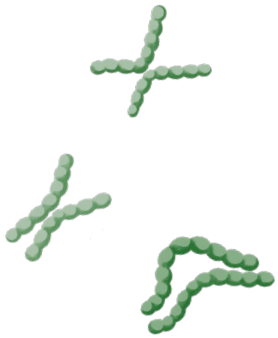


Day 5

Continued

Procedure

15 Tell students that pharmaceutical companies understand that the barriers to treatments are complicated; therefore, they often provide grant funding to help patients with SCD gain access to treatments. Propose the following scenario to students: If a pharmaceutical company provided $500,000 to be used within the next year, and you were advocating for the funds to be spent appropriately, what are a few key factors you would include in your grant proposal (request for funds) to address the inequities people with SCD face in gaining access to high-quality, continuous healthcare? Tell students they will address the following questions in their grant proposal and pause to see if students have questions:

a. How would you ensure this money is used appropriately to maximize the benefit to underserved communities?

b. How would you ensure cultural humility plays a role in how the funds are spent?

c. How could the money be spent to improve communication between clinics and patients with sickle cell disease?

Whole Group (5 min)

When students have completed their partner discussions, ask for student volunteers to share their ideas as a whole class.

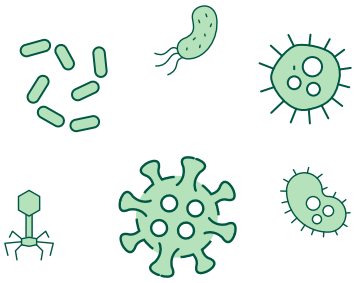
Day 6

Procedure

LEARNING OUTCOMES

Students will be able to:

Make predictions involving the impact of the relationship between bacteria and phages on health, the environment, or food production.



Whole Group (5 minutes)

Ask students to list the different types of defenses covered, recording what they come up with on the board and filling in what they do not come up with.

a. preventative absorption

b. inhibiting DNA injection

c. silencing by lysogen repressor

d. DNA cleavage by restriction enzymes

e. toxin-antitoxin systems/abortive infection

f. assembly interference

g. CRISPR-Cas9

Individual Work (30 minutes)

Give students the *Microbe Phage Scenario Articles*. Assign students one of four scenarios involving the relationship between bacteria and phages, and having a direct effect on either human health, the environment, or food production. Tell students they will address the following in their **Toolkit**:

a. Identify the problem in the scenario.

b. Decide which they would want to see thrive, the bacteria or the phages, in order to improve conditions.

c. Explain how the bacteria would try to defend against the phages using at least three defense mechanisms, one needing to be CRISPR-Cas9.

d. Defend their argument in Task b with a justification as to why the bacteria or the phages should be favored.

e. Predict the impact on the scenario and potential evolutionary consequences.

f. Predict the impact on humans of different cultures or socioeconomic status.

Continues next page >

Day 6

Continued

Procedure

Whole Group (10 minutes)	
1	Tell student groups to briefly share what they learned about each scenario, recording information on the board.
2	Remind students that as they have learned about bacterial defense, they should also focus on careers involving these microbes. Give students an opportunity to read the *scientist profile on Kevin Soloman*, focusing on what he does in his field and what might be most relatable or what resonates most with the students. They should log their thoughts in the **Toolkit**: *Based on the career profile in this lesson, what does this tell you about the types of people that do science? What did you find most relatable? If time permits, you may ask students to share their thoughts.*

National Standards

Next Generation Science Standards

LS1-2 From Molecules to Organisms: Structures and Processes

Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.

LS1.A: Structure and Function

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. (HS-LS1-1)

LS3.A: Inheritance & Variation 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 as-yet known function. (HS-LS3-1)

Science and Engineering Practices

Developing and Using Models

Develop, revise, and/or use a model based on evidence to illustrate and/or predict the relationships between systems or between components of a system.

Develop and/or use multiple types of models to provide mechanistic accounts and/or predict phenomena, and move flexibly between model types based on merits and limitations.

Career and Technical Education (CTE)

A1.1

Use data to explain how biotechnology fields such as pharmaceuticals, agriculture, diagnostics, industrial products, instrumentation, and research and development are impacting human life.

A1.3

Recognize the role of innovation in creation of emerging biotechnology careers, including those in nanotechnology, biofuels, and forensics.

Continues next page >

National Standards

CTE

Continued

A1.6
Explore and outline the various science and non-science fields and careers associated with biotechnology.

A2.4
Understand the critical need for ethical policies and procedures for institutions engaged in biotechnology research and product development.

A3.3
Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.

A3.5
Predict outcomes of DNA and protein separation protocols.

A4.6
Distinguish between prokaryotic cells, eukaryotic cells, and viruses.

A7.0
Understand the function of regulatory agencies for the biotechnology industry and the lasting impact of routine laboratory and communication practices on product development and manufacturing.

4.1
Use electronic reference materials to gather information and produce products and services.

4.3
Use information and communication technologies to synthesize, summarize, compare, and contrast information from multiple sources.

4.5
Research past, present, and projected technological advances as they impact a particular pathway.

5.6
Read, interpret, and extract information from documents.

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National Standards

CTE *Continued*	**7.4** Practice time management and efficiency to fulfill responsibilities.
	7.8 Explore issues of global significance and document the impact on the Health Science and Medical Technology sector.
	9.5 Understand that the modern world is an international community and requires an expanded global view.

Bacterial Defense Strategies Capture Sheet

ANSWER KEY **Do not share with students**

Directions

You will design a defense system for a type of bacteria focusing on ONE phase in the phage life cycle and that will stop the virus at this phase. You should be able to explain the following:

1. How could a type of bacteria use a weapon or shield to specifically prevent infection or future attack on other bacteria?

Each phase of the phage life cycle is targeted: preventative absorption, inhibiting injection, lysosome inhibition, CRISPR, Restriction enzymes, assembly interference, toxins.

2. Give your defensive or offensive mechanism a unique and creative name or analogy.

Answers will vary (examples: The Impenetrable Fortress, Excalibur, etc.)

3. With your partner, you will model how the defense system works to a group of students. With the materials provided, create a model or prototype that will help in your explanation. For instance, a pipe cleaner inside a cup might be used to model the phage genetic material (pipe cleaner) inside the capsid (cup). Take a photo of your prototype and place it in the box below.

(Photo inserted here.)

Continues next page >

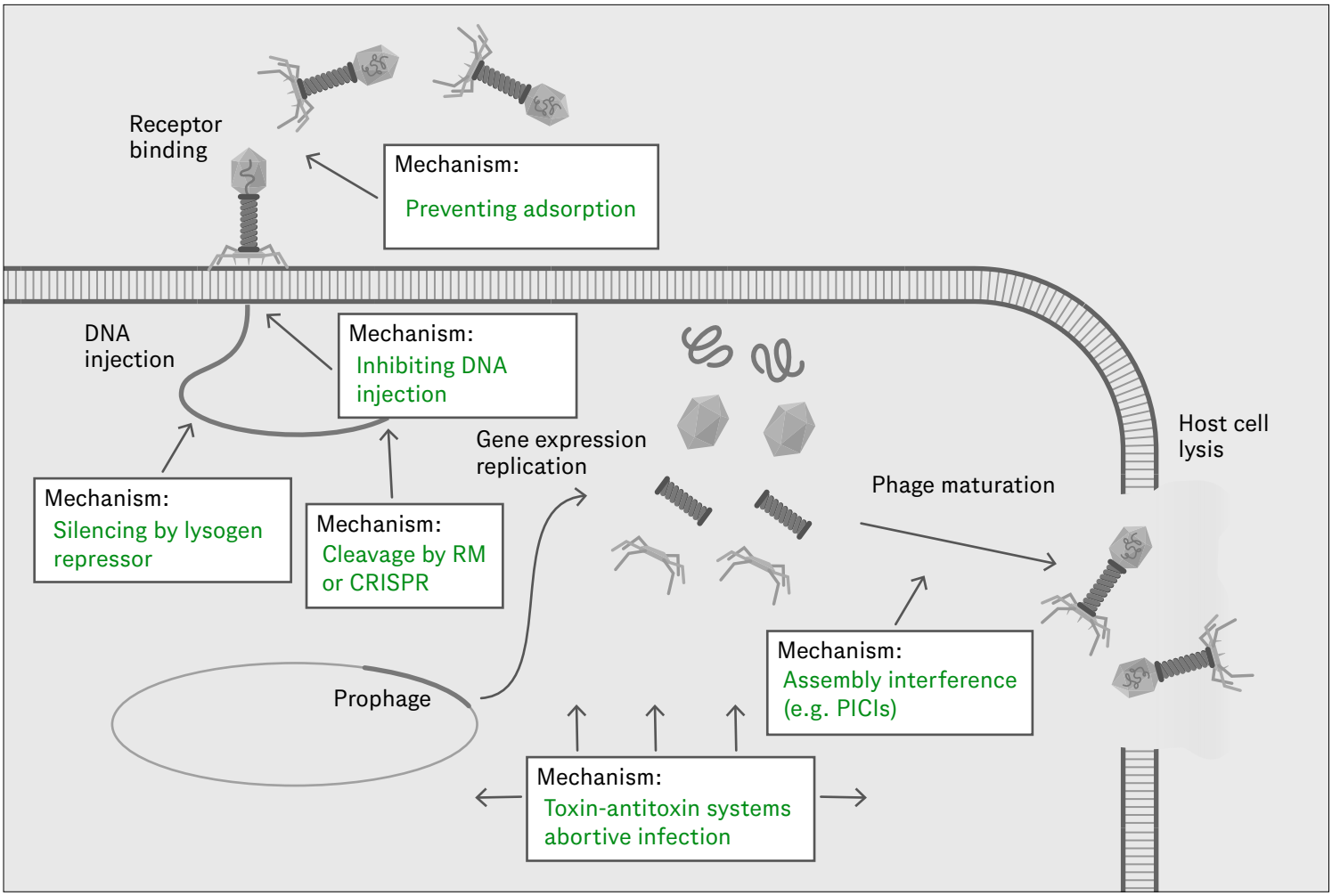
Bacterial Defense Strategies Capture Sheet

ANSWER KEY **Do not share with students**

Continued

4. As each student group shares the bacterial defense against phages, write the name and description on the diagram. Additionally, transfer the names of these mechanisms to your **Toolkit**.

Bacterial Defense Against Phages



Restriction Enzymes Capture Sheet

ANSWER KEY **Do not share with students**

Directions
Watch the Restriction Enzymes video from Cold Spring Harbor Laboratory's DNA Learning Center, and answer the following questions about restriction enzymes.

1. Name one scientist who worked on restriction enzymes.

Werner Arber, Daniel Nathans, Hamilton Smith

2. What are the phases of the phage life cycle?

Bacteriophage adsorbs to the outside of the host, injects genetic information into the host cell, uses ribosomes to transcribe viral proteins, and then the virus is assembled inside the host cell.

3. Once in a while a "surprise" result would occur. What was that surprise?

The cells would survive, due to an enzyme that cut up the phage DNA after the phage got into the cell.

4. Why were they called restriction enzymes?

They restrict the replication of the phages.

5. Why is EcoR1 named as it is?

The restriction enzyme came from the bacteria, *E. coli*, R is the strain it was isolated from, and 1 is because it's the first one isolated.

Continues next page >

Restriction Enzymes Capture Sheet

ANSWER KEY **Do not share with students**

Continued

6. What are the overhanging ends called when EcoR1 cuts the DNA?

Sticky ends

7. How can a jellyfish gene, such as GFP, be inserted into a mouse cell?

The restriction enzymes can be used to cut the gene out of the jellyfish, and to cut a space in the mouse genome. Ligase can be used to fuse the two together. This is an oversimplification (as stated in the video).

8. What are similarities between the restriction enzyme bacterial defense strategy and the human immune system?

Restriction enzymes act like the human immune system in targeting and destroying specific invaders so they cannot replicate.

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Restriction Enzymes Capture Sheet

ANSWER KEY **Do not share with students**

Continued

9. In small groups, select one product to focus on in your field of choice (health, food, or environment), and note that in the table below. Research how restriction enzymes have been applied to that product.

Answers will vary with the product chosen. Below are examples.

	Product	Impact and Benefit	
A	Human Health: Human insulin produced by *E. coli*	What group of people does this impact the most?	People with diabetes, people without healthcare
		How are the restriction enzymes used?	RE cut the human insulin gene out of the human cell and cut the plasmid in the bacteria. The insulin gene is ligated into the bacterial plasmid and transformed back into the bacteria.
		What are the benefits?	We can make insulin much more easily.
		What are the potential risks?	Scientists must be cautious when extracting the insulin gene from the bacteria, making sure the correct sequence is inserted. Also, not everyone can afford insulin as currently three companies control 90% of global insulin production (and are charging too much for it).
B	Food Production: GM crops, example: corn	What group of people does this impact the most?	Farmers, consumers
		How are the restriction enzymes used?	RE are cut sequences from bacteria, *Bacillus thuringiensis*, and inserted into corn to produce toxins that kill pests that try to eat it.
		What are the benefits?	Resist pests and tolerate herbicides
		What are the potential risks?	Concerns with genes from GM crops crossing with non-GM crops, farmers becoming dependent upon herbicides
C	Environment: Bioethanol from genetically engineered algae	What group of people does this impact the most?	People in need of alternative fuel sources; only certain people may be able to afford these potentially more expensive fuel sources because new technologies tend to cost more.
		How are the restriction enzymes used?	RE is used to insert biofuel producing genes into the algae.
		What are the benefits?	Creating bioethanol is a greener way to produce fuels.
		What are the potential risks?	Ecological risks posed by cultivation of GE algae are largely unknown.

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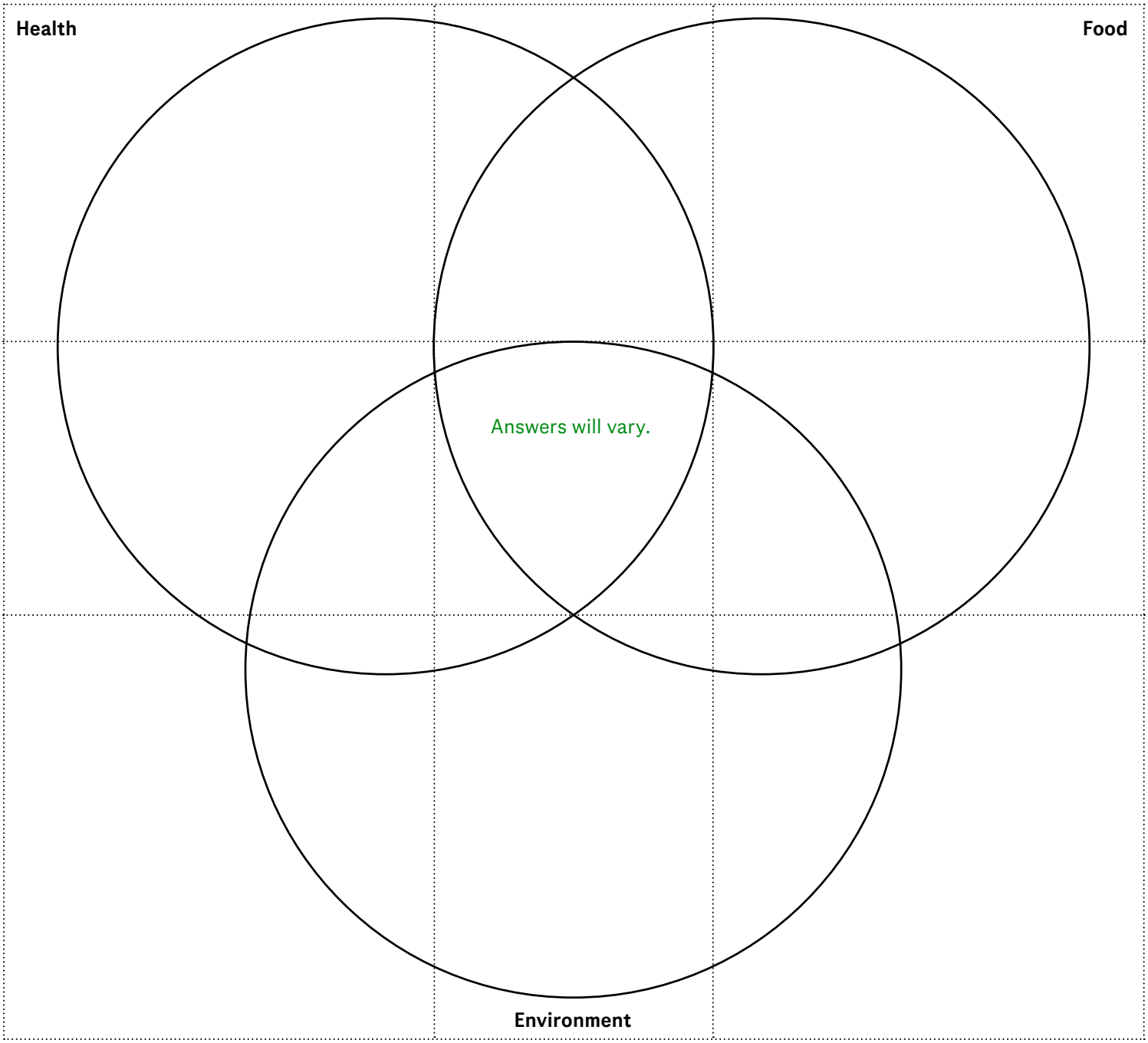
Restriction Enzymes Capture Sheet

ANSWER KEY **Do not share with students**

Continued

10. Use research from others to note similarities and differences between the three areas using the Venn diagram below.



History of CRISPR-Cas9 Capture Sheet

ANSWER KEY **Do not share with students**

Directions
Use the Background Reading: History of CRISPR-Cas9 to answer the following questions.

1. As you read through the history of CRISPR-Cas9, how many scientists did you notice contributed to this research (assume that on average, about seven scientists collaborated on a paper with each scientist mentioned)? Does this number surprise you?

I estimated 14, and no because it is extremely complicated.

2. Approximately how many years of research did it take to go from discovering the CRISPR sequence to developing it as a tool for genome editing?

18 years (1993–2011)

3. Name one scientist who was studying CRISPR-Cas9 out of pure curiosity.

Jennifer Doudna

4. Of all the scientists working on CRISPR-Cas9, which were focused on editing the human genome?

George Church and Feng Zhang

5. In your own words, describe what CRISPR-Cas9 is as a bacterial-acquired immunity.

It can create a "memory" of how to beat a disease.

6. Explain how CRISPR-Cas9 could be used to modify the genome of an organism.

It could be used to edit damaged cells or omit an unwanted gene.

Continues next page >

History of CRISPR-Cas9 Capture Sheet

ANSWER KEY **Do not share with students**

Continued

7. Put yourself in the shoes of one of the scientists you read about. From that scientist's perspective, write a fictional tweet that this person might post about his or her research, or the reaction that a scientist might have had after discovery. This will be used in an activity tomorrow where other people will try to guess who the scientist is based on the tweet. Below your tweet, write the name of the person who would have tweeted this. You have a 280-character limit.

Answers will vary. Below is an example.

Example Tweet

Tweet "I saw some weird sequences of DNA, had no idea what they were, and didn't make a big deal of it."

Scientist Yoshizumi Ishino

Your Tweet

Tweet Just cloned some CRISPR tech #success

Scientist Virginijus Siksnys

CRISPR-Cas9 Mechanism Capture Sheet

ANSWER KEY **Do not share with students**

Directions

Show your understanding of the CRISPR-Cas9 mechanism by completing the following questions.

1. With a partner, cut each phrase from the far right column and place it in the table to show which component of the CRISPR-Cas9 system it applies to. "Cleaves DNA" has been completed as an example.

Characteristics	Components of the CRISPR-Cas9 system			
	Cas9	PAM	guide RNA	Target
RNA polymerase binds nearby	Cleaves DNA			
Contains functional endonuclease Antigen	Contains functional endonuclease Antigen	3-nucleotide sequence recognized by Cas9	Leads Cas9 to target	RNA polymerase binds nearby
3-nucleotide sequence recognized by Cas9	Sterically inhibits transcription	Designed to complement target		Any sequence near PAM
Sterically inhibits transcription		May be a coding site or regulatory site		About 20 nucleotides long
Designed to complement target				Sequence to be cleaved
About 20 nucleotides long				
Leads Cas9 to target				
Sequence to be cleaved				
Any sequence near PAM				
May be a coding site or regulatory site				

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CRISPR-Cas9 Mechanism Capture Sheet

ANSWER KEY **Do not share with students**

Continued

2. Summarize and explain the components of the CRISPR-Cas9 system and the four steps of how it works (targeting, binding, cleaving, DNA repair).

The first phase of the process is the targeting phase. In this phase, guide RNA (gRNA) leads Cas9 to a DNA complement. Cas9 binds to a three nucleotide sequence called PAM, where it proceeds to unwind the DNA. If the gRNA matches the complement sequence, the binding phase occurs. After binding is finished, the cleaving phase begins. Here, Cas9 cuts the DNA, then the gRNA dissociates from Cas9. The last phase is the DNA repair phase, where mutations are targeted. If the mutations make the protein non-functional, two types of repair could occur. Either non-homologous end joining will deactivate the gene, or scientists can insert a "healthy" copy of the gene during homology-directed repair.

What is Sickle Cell Disease Capture Sheet

ANSWER KEY **Do not share with students**

Directions
Use the FDA approves first test of CRISPR to correct genetic defect causing sickle cell disease video and the What is Sickle Cell Disease Reading to answer the following questions.

1. How are sickle cells different from regular red blood cells? How does this impact blood flow?

Sickle cells bend and can block blood flow, leading to serious problems.

2. What kind of disorder is sickle cell disease (SCD)?

It is a hereditary disease.

3. Worldwide, where is it most prevalent?

Africa, Eastern Asia, India, South America

4. Why and how does SCD cause pain?

The sickle shaped cell blocks blood flow and oxygen to vital organs.

5. How could CRISPR-Cas9 help cure SCD?

If we can correct the mutation, we can cure the disease.

6. Who in the United States is most affected by SCD?

People of African descent

Transcribing and Translating the BCL11A Repressor

ANSWER KEY — **Do not share with students**

Directions

Below is a sequence of DNA from the BCL11A repressor.
Follow the steps to visualize its normal folding pattern.

1. Cut along the solid lines and tape or paste the DNA strand by joining ends together (A tapes to A, B tapes to B, etc.).

2. Using the *mRNA codon chart*, transcribe and translate the underlined DNA sequences below (where AA stands for amino acid).

Sequence A

DNA	Ø	GTCT**GCT**TCCATCCAGACTCCTGACGTT**CTC**GTTCGCAGGGACGTCACGTCCGCACTTGA**TTT**TGCAGCT	A
mRNA		CGA GAG AAA	′
AA		Arg (1) Glu (4) Lys (3)	
DNA	A	CAGGTTTTCTTTTGCCATTTTTTTCATCTCTCTCTCTCTCTCTCCCT**CAT**TCTCTCTTCTCTCTCTCTCC	B
mRNA		GUA	
AA		Val (5)	
DNA	B	C**TAC**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTTAAAAAAAAGC**ATA**GACGGCTCTCCCACAATTCAT	C
mRNA		AUG UAU	
AA		Met (6) Tyr (2)	
DNA	C	**CTG**CCCTGCGCCATCTTTGTATTATTTCTAATTTATTTTGGATGTCAAAAGGCACTGA**AAA**AGATATTTT	D
mRNA		GAC UUU	
AA		Asp (3) Phe (6)	
DNA	D	CTCTGGAGTCTCCT**GAT**TTCTAACCCGGCTCTCCCGATGTGAA**GTT**AGCCGTCGTCCGCCCGCCGCCGCC	E
mRNA		CUA CAA	
AA		Leu (5) Gin (2)	
DNA	E	GCCGCCGCCGCCG**GTT**CCCGCCCCGCAGCCCACCATGTCTCGCCGCA**GTG**AAGGCAAACCCCAGCACTTA	Ø
mRNA		CAA CAC	
AA		Gin (1) His (4)	

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Transcribing and Translating the BCL11A Repressor

ANSWER KEY **Do not share with students**

Continued

3. With your translated amino acid sequence, model protein folding by taping each numbered amino acid together. For example, amino acid 1 with amino acid 1 and so on. This structure represents the complicated, three dimensional structure of the functional repressor protein.

4. Recall that amino acids have different characteristics. Some are polar, some are non-polar, and others have opposite charges that are attracted to each other. In the table below, explain why each numbered amino acid will form a bond with each other. Refer to Sequence A above for the amino acid numbers.

Amino Acid #	Amino Acid Names (abbreviated)	Polar, Non-Polar, or Charged	In general, why do these form bonds the way they do?
1	Arg and Gln	Polar	
2	Tyr and Gln	Polar	
3	Lys (negative) and Asp (positive)	Charged	
4	Glu (positive) and His (negative)	Charged	
5	Val and Leu	Non-polar	
6	Met and Phe	Non-polar	

Continues next page >

Transcribing and Translating the BCL11A Repressor

ANSWER KEY **Do not share with students**

Continued

5. The DNA sequence below is the same as *Sequence A,* representing the BCL11A repressor. We will use this to model how CRISPR-Cas9 cuts. Cut out and tape A to A as you did the previous sequence. Do not tape the amino acids together. Highlight the three-nucleotide-long *PAM* sequences (AGG, CGG, TGG, GGG).

   Note: When reading this piece of DNA, imagine you are flowing down a river as you read from left to right. When discussing positions on the DNA, you can say "that sequence is upstream" or "that site is downstream" from a position of interest.

Sequence B

DNA	Ø	GTCT**GCT**TCCATCCAGACTCCTGACGTT**CTC**GTTCGCAGGGACGTCACGTCCGCACTTGA**TTT**TGCAGCT	A
mRNA			
AA			
DNA	A	CAGGTTTTCTTTTGCCATTTTTTTTCATCTCTCTCTCTCTCTCTCCCT**CAT**TCTCTCTTCTCTCTCTCTCC	B
mRNA			
AA			
DNA	B	C**TAC**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTTAAAAAAAAAGC**ATA**GACGGCTCTCCCACAATTCAT	C
mRNA			
AA			
DNA	C	**CTG**CCCTGCGCCATCTTTGTATTATTTCTAATTTATTTTGGATGTCAAAAGGCACTGA**AAA**AGATATTTT	D
mRNA			
AA			
DNA	D	CTCTGGAGTCTCCT**GAT**TTCTAACCCGGCTCTCCCGATGTGAA**GTT**AGCCGTCGTCCGCCCGCCGCCGCC	E
mRNA			
AA			
DNA	E	GCCGCCGCCGCCGG**TT**CCCGCCCCGCAGCCCACCATGTCTCGCCGCA**GTG**AAGGCAAACCCCAGCACTTA	Ø
mRNA			
AA			

Continues next page >

Transcribing and Translating the BCL11A Repressor

ANSWER KEY **Do not share with students**

Continued

6. Now we will use Sequence B to model how CRISPR-Cas9 can change the function of a protein by cutting the DNA strand. Remember, the guide you will give to Cas9 needs to be an RNA guide. Identify the DNA sequence you will target and transcribe your target to RNA below.

   For example:

Target	A A C G G T A C G A C G A
gRNA	U U G C C A U G C U G C U

   Your Turn: (make sure your target sequence is somewhere that will affect the protein folding and near an NGG site).

Target	Answers will vary.
gRNA	Answers will vary, but should be the complementary sequence.

7. Write the gRNA sequence you developed above into the *CRISPR-Cas9 Model.* Follow the directions on that page to model how CRISPR-Cas9 scans for matching target sites.

8. Recall from previous activities that the cell attempts to repair DNA that has been cut. However, in the attempt to repair, random nucleotides are introduced.

   For the purpose of this activity, we will assume that the random mutation introduced is ATT.

   Without transcribing and translating this shifted DNA sequence with the added ATT, predict how this insertion would impact the structure and folding of the modified protein.

   a. What does ATT code for?

   ATT codes for the stop codon.
   This signals to halt protein systenths.

   b. How will this affect the overall protein?

   The protein would terminate prematurely at this point.

   c. How will this affect someone that has sickle cell disease?

   The stop codon would prematurely stop protein synthesis, causing the protein to fold incorrectly, and thus stop the sickle cells from being produced.

Barriers to CRISPR-Cas9 Therapies Capture Sheet

ANSWER KEY **Do not share with students**

Directions
Read the following, then answer the questions on the next page about approaches to overcoming barriers to treatments.

According to the CDC, SCD affects approximately 100,000 Americans. It has a higher percentage in those whose ancestors came from sub-Saharan Africa, South America, the Caribbean, Central America, Saudi Arabia, India, and Mediterranean countries, such as Turkey, Greece, and Italy. SCD affects 1 out of 365 Black or African-American births in the United States. This is a significant number of people who need access to treatments for SCD. However, there are significant barriers to gene therapies. For example, they generally cost around $10,000 per month. Read more about the economic barriers to gene therapies in the article *Paying for CRISPR Cures: The Economics of Genetic Therapies* from Innovative Genomics Institute. Also, the number of SCD treatment centers in the United States is far fewer than those available for other diseases, such as hemophilia (a blood clotting disease that affects 20,000 people in the United States). The centers that do exist are poorly coordinated with primary doctors and are underutilized. Researchers concerned with and advocating for underrepresented groups participating in clinical trials for new therapies, such as CRISPR, have found that empathy and humility along with understanding the cultural and historical factors at play are key to patient involvement, awareness of, and access to new therapies.

Almost 25 years ago, the National Institutes of Health (NIH) issued a policy requiring that all NIH-funded clinical research include women and minorities, but there are still reports of failure to enroll minorities in clinical trials. Ineffective communication, lack of trust in research, and practical factors, such as time commitments and awareness of available treatments and clinical trials, all play a role in recruiting minority patients.

Researchers have found that fostering cultural humility can make a difference. Cultural humility is the commitment to self-evaluation and valuing unique and diverse populations and their characteristics. It requires openness, self-awareness, and being egoless. When researchers consider the culture and beliefs in the community and when they develop empathy for and engage in the community, it increases community knowledge and awareness of treatments. Recruitment for participation in new treatments need to be tailored to the community and communicated in a way that engages with the culture of the group.

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Barriers to CRISPR-Cas9 Therapies Capture Sheet

ANSWER KEY **Do not share with students**

Continued

1. Why do you think it is important to include underrepresented groups in clinical research?

Research needs to reflect the diversity found in the population to ensure safe medical treatment for all groups because different people can react differently to therapies.

2. What role does cultural humility play in helping patients with SCD gain access to new treatments?

Cultural humility is important to maintain an open mind and awareness of the diversity and differences between the researchers and community.

3. Some pharmaceutical companies understand the barrier to treatments are complicated. They provide grant funding to help patients with SCD gain access to treatments. A pharmaceutical company provided $500,000 to be used within the next year and if spent appropriately, the funding would continue for another year. If you were advocating for the funds to be spent appropriately, what are a few key factors you would include in your grant proposal (request for funds) to address the inequities people with SCD face in gaining access to high-quality, continuous healthcare?

   a. How would you ensure this money is used appropriately to maximize the benefit to underserved communities?

   Answers will vary.

   b. How would you ensure cultural humility played a role in how the funds were spent?

   Answers will vary.

   c. How could the money be spent to improve communication between clinics and patients with sickle cell disease?

   Answers will vary.

FUTURELAB+

Bacterial Defense Strategies Capture Sheet

Directions

You will design a defense system for a type of bacteria focusing on ONE phase in the phage life cycle that will stop the virus at this phase. You should be able to explain the following:

1. How could a type of bacteria use a weapon or shield to specifically prevent infection or future attack on other bacteria?

2. Give your defensive or offensive mechanism a unique and creative name or analogy.

3. With your partner, you will model how the defense system works to a group of students. With the materials provided, create a model or prototype that will help in your explanation. For instance, a pipe cleaner inside a cup might be used to model the phage genetic material (pipe cleaner) inside the capsid (cup). Take a photo of your prototype and place it in the box below.

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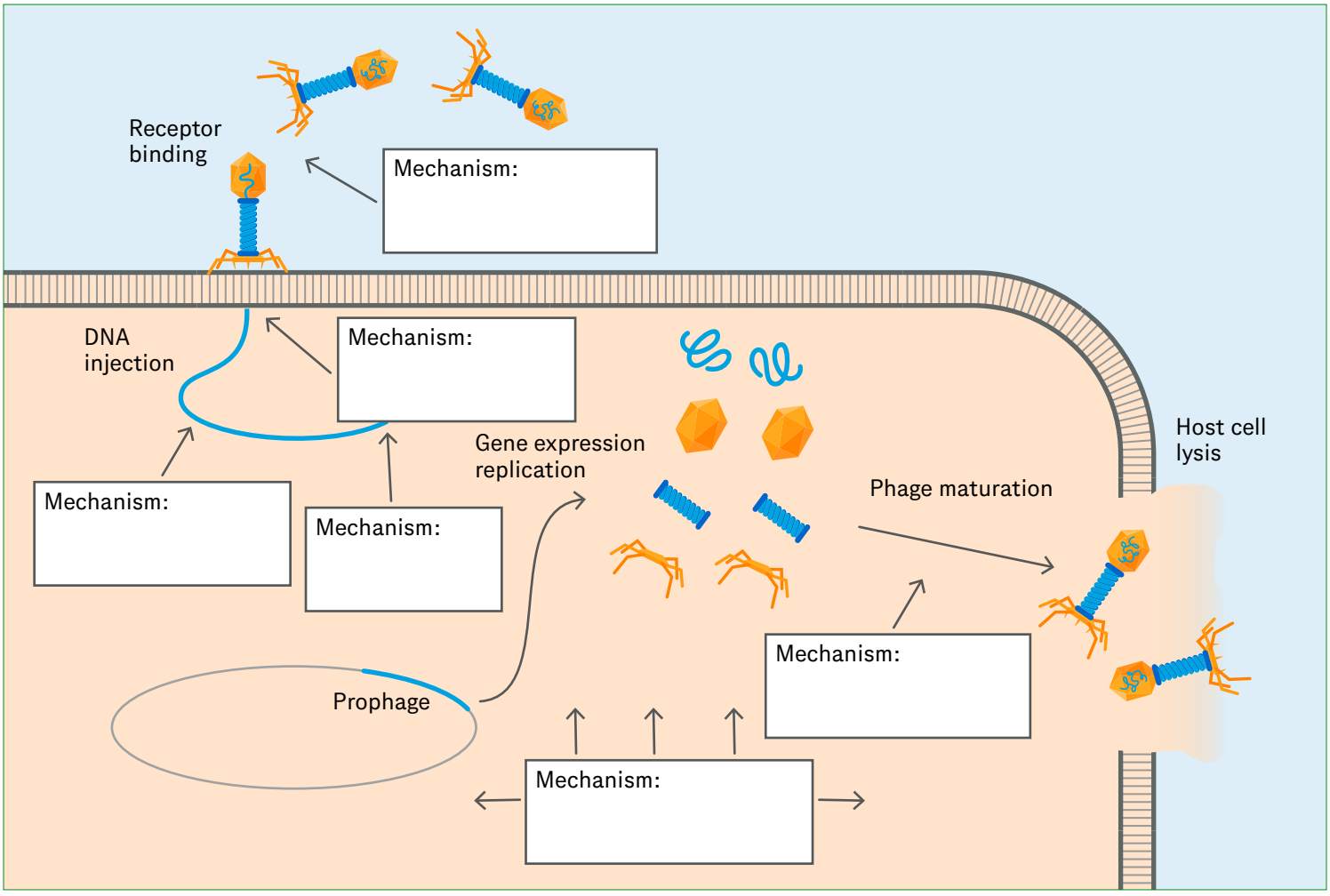
Bacterial Defense Strategies Capture Sheet

Continued

4. As each student group shares the bacterial defense against phages, write the name and description on the diagram. Additionally, transfer the names of these mechanisms to your **Toolkit**.

Bacterial Defense Against Phages



FUTURELAB+

Career Profile

Assistant Professor

Kevin V. Solomon, PhD
University of Delaware's
Chemical and Biomolecular
Engineering Department

What is your current role, and how did you get there?

I am a professor of Chemical & Biomolecular Engineering at the University of Delaware where we engineer bacteria to produce medicines, fuels, and materials. After getting my undergraduate degree in bioengineering, I used synthetic biology tools to work on metabolism with *E. coli* bacteria. From there, I studied fungi and other microbes. I really enjoy the freedom and ability to build things that address problems and am fascinated by the things that biology can do. Simple microorganisms can do so much including make basic medicines (e.g. penicillin), foods, and even fix cracks in concrete (when engineered to do so).

What skills do you use on a daily basis?

I interpret data, forming connections between disparate pieces of information, by reading scientific journals, programming computers, and even just making simple plots. I also need to communicate this information clearly to others, without jargon, such that others may benefit.

What's most fulfilling about your job? What's most challenging?

The most fulfilling part of my job is the freedom to work on jobs that I choose and help students learn to be similarly independent. The most challenging aspect is how to pick the 'right' kind of problems to work on—some problems are harder than others and may be more difficult to solve.

What was your favorite subject in high school, and why did you love it?

My favorite subject was chemistry—I found it fascinating that simple innocuous items could be changed into life saving drugs or high energy explosives by understanding how atoms worked and making appropriate calculations.

What is your most used phone app?

Youtube—there's a lot of great content to teach me about the world (e.g. CrashCourse, Armchair Historian, VSauce) as well as "fun" videos with wacky pranks, music videos, etc.

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Restriction Enzymes Capture Sheet

Directions

Watch the Restriction Enzymes video from Cold Spring Harbor Laboratory's DNA Learning Center, and answer the following questions about restriction enzymes.

1. Name one scientist who worked on restriction enzymes.

2. What are the phases of the phage life cycle?

3. Once in a while a "surprise" result would occur. What was that surprise?

4. Why were they called restriction enzymes?

5. Why is EcoR1 named as it is?

Continues next page >

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Restriction Enzymes Capture Sheet

Continued

6. What are the overhanging ends called when EcoR1 cuts the DNA?

7. How can a jellyfish gene, such as GFP, be inserted into a mouse cell?

8. As a class, consider how the bacterial defense strategy of restriction enzymes is similar to the human immune system, and take a few notes here.

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Restriction Enzymes Capture Sheet

Continued

9. In small groups, select one product to focus on in your field of choice (health, food, or environment), and note that in the table below. Research how restriction enzymes have been applied to that product.

	Product	Impact and Benefit	
A	Human Health:	What group of people does this impact the most?	
		How are the restriction enzymes used?	
		What are the benefits?	
		What are the potential risks?	
B	Food Production:	What group of people does this impact the most?	
		How are the restriction enzymes used?	
		What are the benefits?	
		What are the potential risks?	
C	Environment:	What group of people does this impact the most?	
		How are the restriction enzymes used?	
		What are the benefits?	
		What are the potential risks?	

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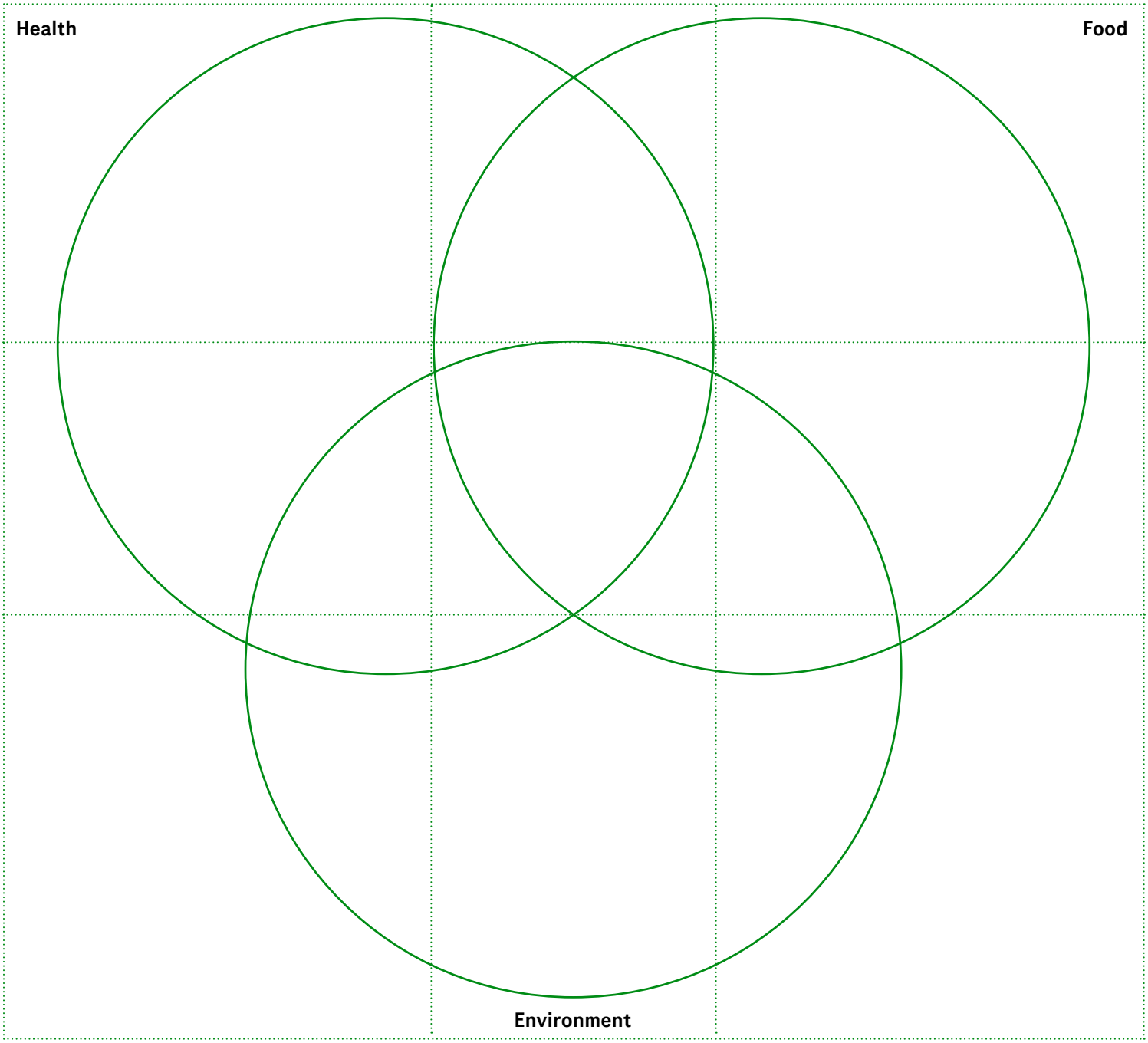
Restriction Enzymes Capture Sheet

Continued

10. Use research from others to note similarities and differences between the three areas using the Venn diagram below.



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Background Reading: History of CRISPR-Cas9

Introduction

CRISPR-Cas9 is the hottest tool used in genome editing, and provides a way to change an organism's DNA. Information can be added, deleted, or modified in the genome to obtain a desired phenotype. Think of all the applications that come to mind as to how this tool can be used to benefit human life—treat or eliminate disease, create more efficient energy sources, or to create ways to have a more sustainable and positive impact on the environment. These are exciting and promising times in genome editing with CRISPR-Cas9, but there are limitations to its use as well as ethical questions that need to be addressed as this technology develops. In this lesson, you will learn about the history of CRISPR-Cas9, discover the specific use of CRISPR-Cas9 in genome editing, and perform a laboratory investigation using CRISPR-Cas9. You will also have the opportunity to explore the applications and ethics associated with using this technology in human cells.

The History of CRISPR-Cas9

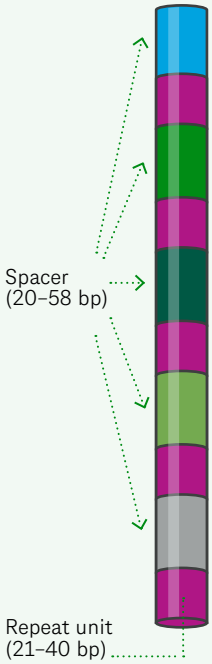
If you have ever eaten yogurt or cheese, you have eaten CRISPR-Cas9. Most people hear CRISPR-Cas9 and think of editing genes, but not many know that it is found in the simplest of microbes and has been around for a long time. The history behind this technology is a fascinating story of how scientific progress takes place. "[The] human stories behind scientific advances can teach us a lot about the miraculous ecosystem that drives biomedical progress—about the roles of serendipity and planning, of pure curiosity and practical application, of hypothesis-free and hypothesis-driven science, of individuals and teams, and of fresh perspectives and deep expertise." From the discovery of a puzzling sequence of DNA in bacteria, to producing yogurt, to developing a tool through synthetic modifications, the story of CRISPR-Cas9 technology is one of evolution, natural history, and technological breakthrough. Below is an outline of the history of CRISPR- Cas9 and how it is used today.

1987: Strange Observations

Even though CRISPR-Cas9 technology has exploded in recent years, the first hint of CRISPR-Cas9 existence goes back to 1987 when Yoshizumi Ishino, a Japanese microbiologist, sequenced the genome of *E. coli* and noted an area of DNA with short repeated nucleotides, spaced by seemingly random DNA sequences, or spacers, as seen in the figure at the top of the next column. Not much was made of it and his team published, "the biological significance of these sequences is unknown."

Microbiologist Yoshizumi Ishino

The area of repeats and spacers Ishino noted in *E. coli* DNA.



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Background Reading: History of CRISPR-Cas9

Continued

1993–2005: Why the name CRISPR?

Six years later at the University of Alicante, 28-year-old nature-loving microbiologist Francisco Mojica was studying a salt-tolerant bacterial strain in the Mediterranean marshes of Santa Pola, Spain; the same town where he grew up. He combed the genome of the bacteria looking for clues as to why they could survive in such a high-salt environment. In doing so, he saw a similar type of random sequences of DNA spaced by repeat units that Ishino saw, and was so fascinated with it that he devoted the next decade of his career to understanding it. Watch a *3 Minute Interview with Dr. Mojica*.

Meanwhile, Ruud Jansen of Utrecht University in the Netherlands, found genes in bacteria with these spacer-repeats that were absent in bacteria without the spacer-repeats. Knowing that Mojica was studying the spacer-repeats, he contacted him, asking him what to call that region so that he and others working on these genes could communicate more efficiently. Together, they coined the term *CRISPR*, or *clustered regularly interspaced short palindromic repeats*, for this spacer-repeat region. Jansen called the genes he found *CAS*, or *CRISPR-associated genes*.

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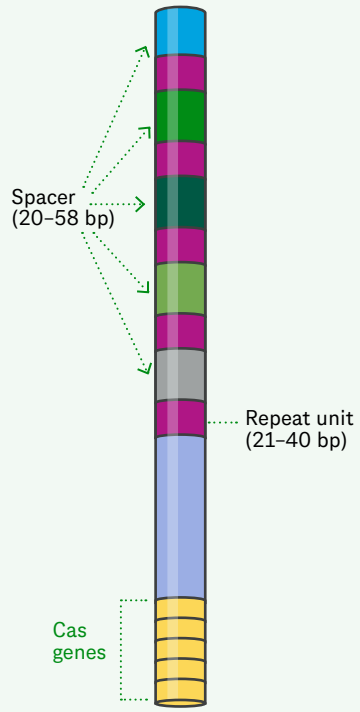
Microbiologist Francisco Mojica

The CRISPR-associated genes along with the spacer-repeat region.



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Background Reading: History of CRISPR-Cas9

Continued

Bacterial Immunity Hypothesis

In 2003, Mojica decided to focus on the spacer region rather than the repeat region. One day on vacation, to escape the heat of the day, he went to his air-conditioned lab and decided to perform a "BLAST," or match search, for these sequences with all organism sequences in a database. He found the same random DNA sequences in *bacteriophages*, or *viruses* that attack bacteria. And not in just one bacteria, but many. Why would viral DNA be in the bacterial genome? Perhaps it worked similarly to the human immune system. After humans fight a novel pathogen, we have what are called memory cells that stick around in case we get infected with the same pathogen again. Our bodies can quickly fight the secondary infection with these memory cells, without getting sick. What if these "random" sequences were the bacterial "memory" of viral infections of the past? That was Mojica's hypothesis. The bacteria records the sequence associated with the virus (the spacer), separates each virus it was previously attacked by (the repeat unit), and can pull from this memory and attack the virus again if it were to be reinfected.

He excitedly wrote a paper and sent it to *Nature* for review. It was rejected. The editor claimed the information was already known. He tried another publisher, then another. All rejected for one reason or another. After an entire year of review and revision, it was finally published in the *Journal of Molecular Evolution* in 2005. Several other researchers around the world were also hypothesizing without empirical evidence that CRISPR was an adaptive immune system of bacteria. One researcher, Alexander Bolotin of Russia, who published a similar paper just a few months after Mojica, hypothesized that a Cas called *Cas9* may have *nuclease* activity, or the ability to cut DNA. He also found that each viral target sequence has a three-nucleotide section associated with it that he called *protospacer associated motifs,* or *PAM*. But, how did these all work together?

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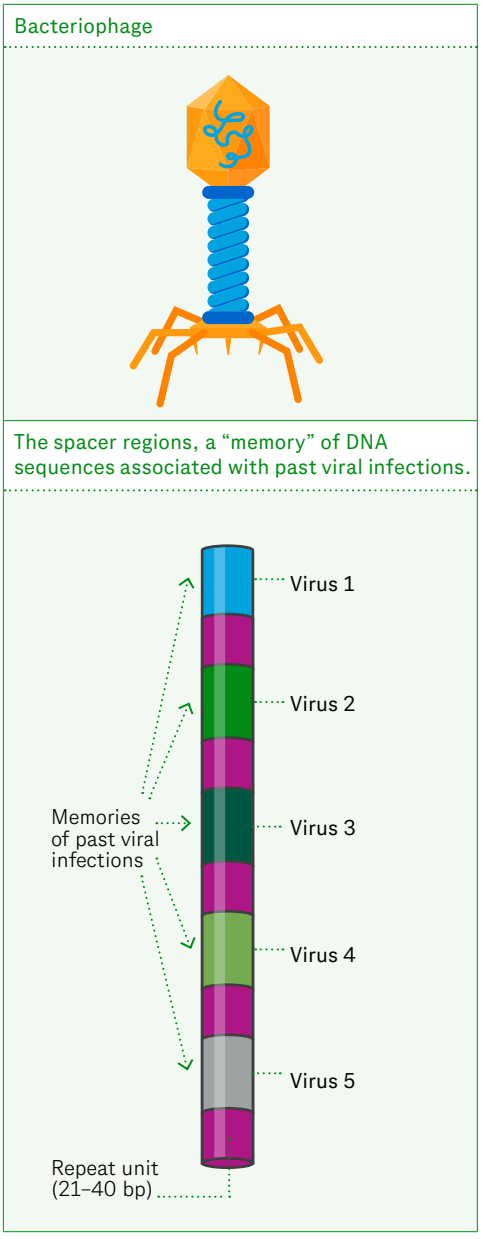

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Background Reading: History of CRISPR-Cas9

Continued

2005–2007: Evidence—For The Love of Cheese

Food scientist, Philipe Horvarth at Rhodia Foods, was working to ensure he had healthy bacteria for cheese and yogurt making—bacteria that could resist viral (phage) infections. This required him to first identify which strains are resistant and to then figure out why. After seeing a CRISPR poster at a Dutch conference in 2002, he noticed the same thing Mojica and other scientists had reported—that the spacers correlated to phage resistance. He and his colleagues, Barrangou and Moineau, tested Mojica's hypothesis that CRISPR was a bacterial adaptive immune system and they produced evidence that it was true. Furthermore, Moineau showed that a Cas protein, called *Cas9*, which had nuclease activity (discovered earlier by Alexander Bolotin), was the only Cas needed for CRISPR to function. There was now evidence that Cas9 was able to cut viral DNA.

2008–2011: How does CRISPR-Cas9 work?

You may have already jumped to the conclusion that CRISPR and Cas9 work together to eliminate viral DNA in bacteria, but how? John van der Oost, who studied extremophiles in his lab in Amsterdam, collaborated with Eugene Koonin, a computational biologist, to prove that the spacer sequences (memories of viruses), are transcribed into RNA pieces called crRNA (CRISPR RNA), also known as guides. These guides lead the Cas9 protein to the target sequence to be cut. If the crRNA guide matched the target sequence, the nucleases in Cas9 would cleave the double-stranded DNA, rendering it inactive. Moineau found that this cleaving activity happened within 3 nucleotides of the PAM sequence on the target DNA, noting its importance in Cas9's activity.

TracrRNA 2011

In her pursuit of a stable and independent research environment, Emmanuelle Charpentier followed her passion leading to work in five countries, seven cities, 10 institutions, and 14 different offices over the past 25 years of her career. These moves allowed her to evolve the way she saw fit as a researcher and each step was made with purpose. As she studied regulatory RNA in bacteria, a small piece that was transcribed from a region near CRISPR caught her attention. She called this missing piece to the CRISPR-Cas9 puzzle tracrRNA, or trans-activating CRISPR RNA. Together, the guide RNA fused to tracrRNA lead the cutting enzyme, Cas9, to the viral DNA. It is then cut and eliminated, as seen in the figure to the right.

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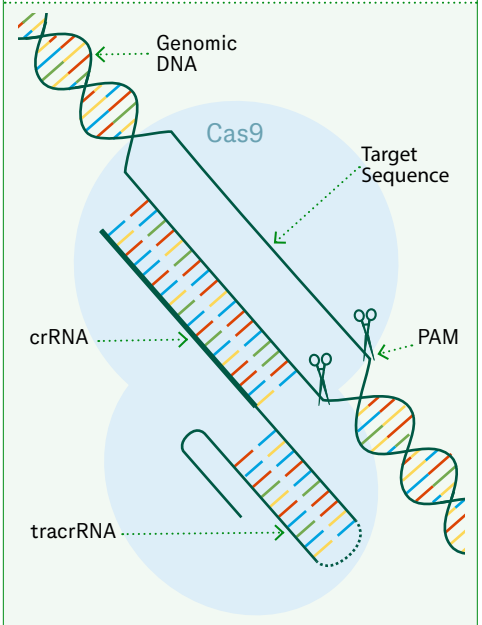
Diagram of CRISPR "scissors"

A diagram of how the guide RNA fuses to tracrRNA to lead Cas9 to the target sequence.



Emmanuelle Charpentier

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Background Reading: History of CRISPR-Cas9

Continued

CRISPR-Cas9 in Vitro 2012

Virginijus Siksnys studied restriction enzymes in Vilnius, Lithuania after getting his PhD from Moscow University. Growing weary of it after 20 years, his excitement for enzymes was rekindled after reading Hovarth, Barrangou, and Moineau's 2007 paper. He felt he would only understand CRISPR-Cas9 if he could reconstitute it in vitro, or outside the cell. He and his colleagues cloned the CRISPR-Cas9 system from one bacteria and placed it into another, and it worked! Impressively, his team also showed that Cas9 could be directed to any target by changing the crRNA guide.

Around the same time, Charpentier gave a lecture about tracrRNA in Puerto Rico. Jennifer Doudna, a structural biologist and RNA expert at UC Berkeley, was in the audience. Doudna, who grew up in Hawaii, was fascinated by the beauty of the environment that surrounded her and was curious about the biological mechanisms underlying all she saw. Influenced by her French teacher at Pomona College to not major in French, but science, she went on to get her PhD from Harvard to study ribosome structure. After hearing Charpentier's lecture, the two joined forces to work on CRISPR-Cas9. They soon discovered that the crRNA and tracrRNA could be combined into a single-guide RNA, or sgRNA.

Both Siksnys and Doudna's groups saw the potential for CRISPR-Cas9 to be a universal tool, publishing papers just a few months apart noting the potential for exploiting the system for wide-range genome editing.

Funnily enough, in 2008, researchers had already come to the conclusion that Cas9 acted as a programmable restriction enzyme that could be used in any organism. Marafini and Sothheimer even began to fill out a patent for the use of CRISPR in eukaryotic cells, but because it lacked experimental demonstration, they abandoned it.

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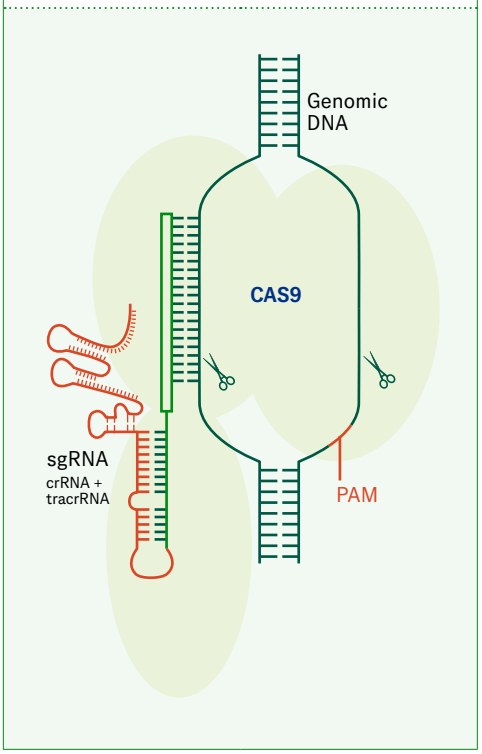
Virginijus Siksnys and Jennifer Doudna

sgRNA is a combination of crRNA and tracrRNA



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Background Reading: History of CRISPR-Cas9

Continued

Mammalian Cells

Feng Zhang got a taste of molecular biology by attending a weekend enrichment course, and was soon working 20 hours a week in a gene-therapy lab near his home in Des Moines, Iowa. He attended Harvard University and became interested in neurobiology when a classmate of his was affected by severe depression. He pursued a PhD in chemistry at Stanford, and with Karl Deisseroth and Edward Boyden, developed a technique called optogenetics, where nerves harboring microbial light-dependent channel proteins could be fired in the presence of light.

Wanting to further develop tools to study neurobiology, he attended a CRISPR-Cas9 talk in February 2011 and was hooked. By April, only two months after being introduced to the technique, he had Cas9 working in human embryonic kidney cells. Further optimizing this tool, he published a paper in October 2012, which became the most cited paper in the field.

George Church, of Harvard, published a paper just a month later with similar findings in human cells. He also proposed using these tools for applications, such as bringing back extinct animals, including the wooly mammoth and Neanderthals, provoking much debate.

Who knew that a bacteria living in a salt marsh in Spain would lead to the most cutting-edge biological tool we have today? We now have the power to rid ourselves of genetic and infectious disease, develop new crops and fuels, and change the life that inhabits the planet. There are so many possibilities. You will soon have a chance to work with this technology in the lab and to explore the opportunities and ethical considerations of such a powerful tool.

Feng Zhang

George Church

Sources:

"The Heroes of CRISPR." 14 Jan. 2016, *The Heroes of CRISPR.* Accessed 4 Jul. 2019.

"History of CRISPR-Cas from Encounter with a Mysterious…—NCBI." *History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology.* Accessed 4 Jul. 2019.

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History of CRISPR-Cas9 Capture Sheet

Directions
Use the Background Reading: History of CRISPR-Cas9 to answer the following questions.

1. As you read through the history of CRISPR-Cas9, how many scientists did you notice contributed to this research (assume that on average, about seven scientists collaborated on a paper with each scientist mentioned)? Does this number surprise you?

2. Approximately how many years of research did it take to go from discovering the CRISPR sequence to developing it as a tool for genome editing?

3. Name one scientist who was studying CRISPR-Cas9 out of pure curiosity.

4. Of all the scientists working on CRISPR-Cas9, which were focused on editing the human genome?

5. In your own words, describe what CRISPR-Cas9 is as a bacterial-acquired immunity.

6. Explain how CRISPR-Cas9 could be used to modify the genome of an organism.

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History of CRISPR-Cas9 Capture Sheet

Continued

7. Put yourself in the shoes of one of the scientists you read about. From that scientist's perspective, write a fictional tweet that this person might post about his or her research, or the reaction that a scientist might have had after discovery. This will be used in an activity tomorrow where other people will try to guess who the scientist is based on the tweet. Below your tweet, write the name of the person who would have tweeted this. You have a 280-character limit.

Example Tweet

Tweet "I saw some weird sequences of DNA, had no idea what they were, and didn't make a big deal of it."

Scientist Yoshizumi Ishino

Your Tweet

Tweet

Scientist

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CRISPR-Cas9 Mechanism Capture Sheet

Directions

Show your understanding of the CRISPR-Cas9 mechanism by completing the following questions.

1. With a partner, cut each phrase from the far right column and place it in the table to show which component of the CRISPR-Cas9 system it applies to. "Cleaves DNA" has been completed as an example.

Characteristics	Components of the CRISPR-Cas9 system			
	Cas9	PAM	guide RNA	Target
RNA polymerase binds nearby	Cleaves DNA			
Contains functional endonuclease Antigen				
3-nucleotide sequence recognized by Cas9				
Sterically inhibits transcription				
Designed to complement target				
About 20 nucleotides long				
Leads Cas9 to target				
Sequence to be cleaved				
Any sequence near PAM				
May be a coding site or regulatory site				

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CRISPR-Cas9 Mechanism Capture Sheet

Continued

2. Summarize and explain the components of the CRISPR-Cas9 system and the four steps of how it works (targeting, binding, cleaving, DNA repair).

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CRISPR-Cas9 Interview

Directions

As a group, select which role you would like to play (the interviewer, Doudna, Charpentier, or Siksnys), then go through the interview with each student reading their part.

Key

#	Interviewer
EC	Emmanuelle Charpentier
JD	Jennifer Doudna
VS	Virginijus Šikšnys

1 To get started, back in 2012, you published your respective landmark papers about CRISPR-Cas9—papers that provided the pathway for science to manipulate the genetic makeup of, really, all life. At the time, the potential of this technology was just being imagined. Now, six years later, what do you see as its most promising applications?

EC The range of CRISPR-Cas9 technology applications has quickly been adapted by the scientific community in a very broad manner. This shows how versatile and powerful the technology is, and it's still developing extremely fast with new applications being published almost weekly. I think the most interesting applications are in the medical field, and hopefully we'll see the technology being successfully applied to correcting more genetic disorders.

VS These days, CRISPR-Cas9 applications span very different fields, from basic biology, to biotechnology and medicine. I agree with Emmanuelle that probably the most promising applications will be in biomedicine and human disease. We are looking forward to the application of CRISPR-Cas9 gene editing for curing inherited genetic disease in humans, and such experiments are going on in different labs across the world.

JD I'll make two additional points. One is that in terms of clinical applications, I'm most excited about using CRISPR-Cas9 to correct disease-causing mutations in blood diseases, such as sickle cell disease. Many groups and companies are trying to do this now, and there's a very real possibility that there will be a genetic cure for this disease in the foreseeable future. That would be an incredible advance. It foretells what may come for other diseases in which there's a single-gene cause and you can envision correcting it using genome editing. The big challenge there is how to deliver gene-editing molecules into a tissue. Blood diseases have been one of the early targets because of the potential to do the delivery ex vivo, in cells that are removed from a patient and then put back into them after the mutation has been corrected. My other comment is that if you think globally about the impact of genome editing on human society, the bigger impact is likely to be in agriculture. We all have to eat, right? The potential to make genetic changes to plants that will protect them from drought or introduce other beneficial traits has gotten much easier now that we have CRISPR-Cas9 technology.

VS Yes, these agro-biotech applications will come a bit quicker than applications in the biomedical field.

2 Do you like working with CRISPR-Cas9?

JD Yes, it is very interesting.

3 Let's talk about your pathways as researchers. Dr. Doudna, you've mentioned that your love of science really took root in large part because of a high school chemistry class. What was it about that class that made science so compelling for you?

JD I had a chemistry teacher, Miss Wong, when I was in tenth grade at Hilo High School in Hilo, Hawaii. She taught us that science is about discovery. It's not about memorizing facts in the textbook. It's about asking questions about the natural world and coming up with ways to figure out answers, and I absolutely love that. I love the idea that you're posing and trying to answer a series of questions and learning things that potentially nobody ever has understood about the natural world. I still feel very excited about the way science works. It's an incredibly fun process of discovery. It's not without frustration, for sure, but that's part of the process, and it's something that I learned when I was in that chemistry class.

4 And Dr. Šikšnys, what factors influenced your career decisions and interest in science?

VS My path to science was similar to Jennifer's because I had a really great chemistry teacher in high school. She boosted my interest in chemistry. She trusted me with the key to the chemistry laboratory where I was allowed to do some simple experiments. And Lithuania had a system of chemistry competitions, called Olympiads. Encouraged by the teacher, I started to actively participate in these competitions, and there was no question what I would do when I graduated from high school.

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CRISPR-Cas9 Interview

Continued

5 Dr. Charpentier, you have talked about how resourceful you needed to be in overcoming funding challenges for your research. For example, you've had to establish your lab multiple times as you've moved from university to university to advance your work. Funding aside, what are some of the other challenges that each of you has faced?

EC I've had to adapt quickly to new places, new colleagues and new funding situations. Another challenge was to rapidly establish my labs, which is linked to the challenge of hiring the right people and bringing the two together in the right way. And it's always a challenge to find the right scientific niche and develop research projects within the frame of topics that are of interest to the scientific community and that could have potential impact beyond fundamental research.

JD I do a lot of work with graduate students and undergraduate students because I'm at a large public university—the University of California, Berkeley. And so one of the challenges is working with people from all over the world who come to the university to learn and to do science together, and facing not only the intrinsic challenges of doing science, but also trying to learn enough about each person I'm working with that I can find out what they're really good at, what they enjoy doing, then help them get onto projects that really maximize their skills.

VS One of the challenges that I sometimes encounter is to convince local funding agencies that doing basic research is very important, because these days there is a clear bias toward applied science. Funding agencies ask you about innovation or new technologies, but usually these innovations and new technologies emerge from simply trying to answer very basic biological questions. This is important to point out.

6 Turning to the public's understanding of gene editing generally, what are the most dangerous misconceptions that you feel need to be corrected?

EC Overall, I'm satisfied that the public understands the benefits of the technology, that it's transformative for research and development and for our own well-being. And actually, the media treat the field very well. There has been some hype with regard to the danger of the technology, some of which was not really justified. But overall, the media have highlighted the benefits and the fundamental research origin and potential of the technology.

VS Fears arise when people encounter new technologies that they do not understand. The same thing happened in the 1980s when genetic engineering started to be introduced to the world. There were fears that scientists would create superbugs that would exterminate people and so on. But now, if you look retrospectively, after 40 years, nothing very terrible happened. In contrast, these genetic engineering methods were used to develop really useful bacteria that produce drugs and provide tools that could improve human health and contribute to the development of society.

7 Who came up with the term CRISPR-Cas9?

JD Francis Mojica.

3 When you started working on the CRISPR-Cas9 gene-editing technology, you were all doing fundamental research in bacterial immune systems. The late paleontologist Stephen Jay Gould once said that life on our planet has always been in the Age of Bacteria. How much of the richness of the bacterial world have we sampled, not necessarily just through the gene editing technology, but also through more fundamental studies of the CRISPR-Cas9 genetic sequence itself?

JD Just a tiny fraction of the microbial world has been sampled. Most microbial organisms haven't been investigated by science because they can't be grown in a laboratory. Imagining what's out there in the microbial universe is one of the things I find so fascinating right now about the science we're doing.

In fact, most of the technologies that have been so instrumental in bringing molecular biology into the modern era and serve as the foundation for so many biotechnology companies today come from studying microbes. It's exciting to think about what's in the future as more and more people investigate aspects of the microbial world that haven't been studied yet.

EC We need to continue to support fundamental research in microbiology. It is by studying the diversity of the microbial world that we will continue to find interesting mechanisms that can be exploited to improve the genetic toolboxes that already exist.

VS Actually, CRISPR-Cas9 was discovered as an antiviral defense system in bacteria. But there are many others, including one called restriction-modification system, which was behind the revolution in biology in the 1980s that Jennifer mentioned. I'm sure that further studies of bacteria will lead to new discoveries.

Source: *2018 Kavli Prize in Nanoscience: A Conversation with Emmanuelle Charpentier, Jennifer Doudna and Virginijus Šikšnys*

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What is Sickle Cell Disease Capture Sheet

Directions

Use the FDA approves first test of CRISPR to correct genetic defect causing sickle cell disease video and the What is Sickle Cell Disease Reading to answer the following questions.

1. How are sickle cells different from regular red blood cells? How does this impact blood flow?

2. What kind of disorder is sickle cell disease (SCD)?

3. Worldwide, where is it most prevalent?

4. Why and how does SCD cause pain?

5. How could CRISPR-Cas9 help cure SCD?

6. Who in the United States is most affected by SCD?

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What is Sickle Cell Disease Reading

Directions
Use the following reading to complete the What is Sickle Cell Disease Capture Sheet.

What Is Sickle Cell Disease?

Source: *NIH's Sickle Cell Disease*

Sickle cell disease is an inherited disease, and a life-long illness that affects red blood cells. Normally, red blood cells are disk shaped and flexible, which makes them move easily through blood vessels. In sickle cell disease, the red blood cells are crescent or "sickle" shaped, making them stiff and sticky. They do not flow through blood vessels as well and can block blood supply in specific areas of the body. If blood flow is blocked, it can cause serious pain, as well as stroke, eye problems, and infections.

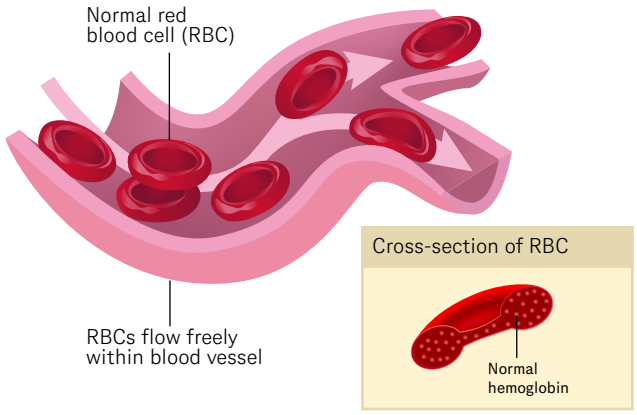
The only current cure for sickle cell disease is a blood and bone marrow transplant. There are some treatments that can reduce symptoms and prolong life, however even with treatment, most people diagnosed have a reduced life expectancy. All major organs in the body (liver, heart, kidneys, etc.) can suffer damage from the abnormal function of the sickle-shaped blood cells.

Sickle cell disease results in abnormal hemoglobin, which is the protein in red blood cells that carries oxygen throughout the body. The abnormal hemoglobin is caused by mutations in the beta-globin gene. People who have sickle cell disease inherit two abnormal beta-globin genes; one from each parent.

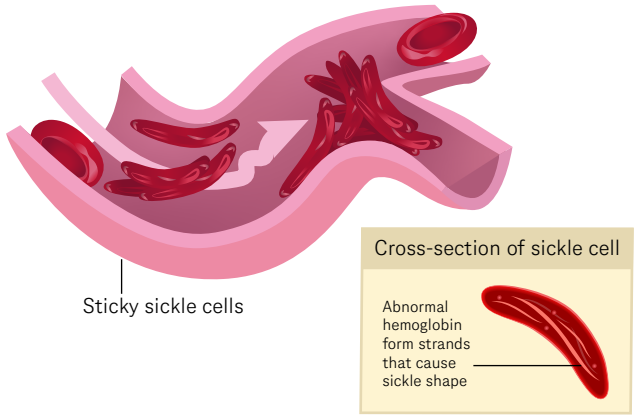
With normal hemoglobin, the red blood cells pick up oxygen from the lungs and carry it through the arteries to all the cells in the body. Once the oxygen has been delivered, the red blood cells return to the heart through the veins—therefore, the veins carry blood with low levels of oxygen. In sickle cell disease, when the blood cells lose their oxygen, they form into the sickle shape. When the blood cells are sickle shaped, they are not as flexible and often stick to vessel walls. This can cause a blockage that slows or stops the flow of blood, impacting the oxygen levels in nearby tissues. The lack of oxygen can cause sudden, severe pain without warning. Most pain attacks require a hospital visit for effective treatment.

Mechanism of a repressor inhibiting transcription

Normal red blood cells



Abnormal, sickled, red blood cells *(sickle cells)*



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What is Sickle Cell Disease Reading

Continued

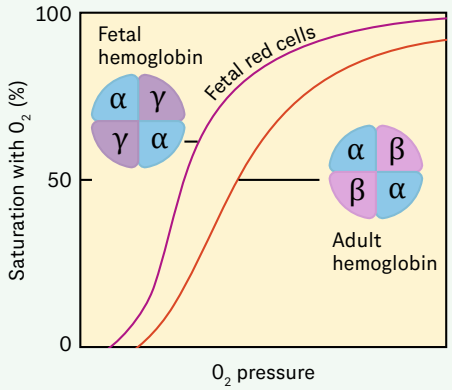
While normal blood cells can live about 90 to 120 days, sickle cells only live for about 10 to 20 days. Circulating blood is filtered through the spleen to remove infections. Sickle cells will often get stuck in this filter and die. The body is always making new red blood cells to replace the old cells, however in sickle cell disease, the body cannot keep up with how fast new cells need to be made. With fewer healthy red blood cells circulating, people often become chronically anemic and have less energy.

Hemoglobin is a protein with four subunits. In adults, the four subunits are two α (alpha) subunits and two β (beta) subunits. In the fetus during gestation, hemoglobin is composed of two α (alpha) and two γ (gamma) subunits—rather than the beta subunits in the adult version. As seen in the graph (right), the composition of the fetal hemoglobin means that it is able to bind more tightly to oxygen at a lower oxygen saturation level. This enables oxygen to move from the mother's bloodstream into the fetus.

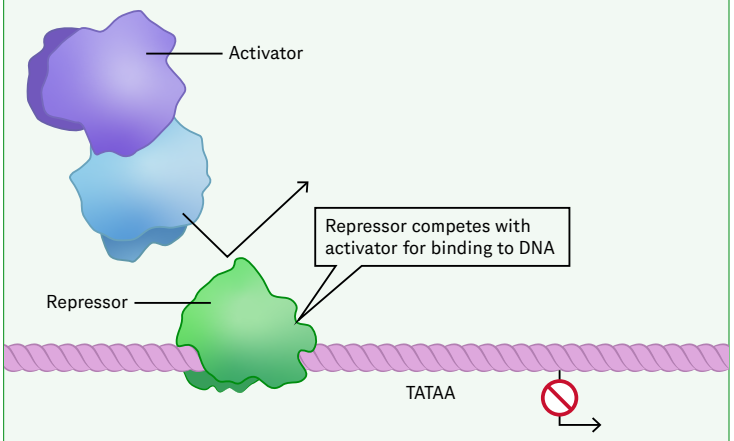
Once a baby is born, the gamma-globin protein stops being made. The cell controls which genes are transcribed, and therefore which proteins are made, using transcription factors. An activator is a transcription factor that increases the transcription of a gene, while a repressor decreases it. In adults, a repressor binds to the DNA sequence for the gamma-globin gene and inhibits its transcription.

Recall that in sickle cell disease, the beta-globin gene carries the mutation. If the gamma-globin repressor were disabled, and transcription of the gamma-globin gene resumed, this subunit could potentially replace the mutated beta-globin gene and alleviate the symptoms of sickle cell disease.

Oxygen saturation of Adult and Fetal Hemoglobin



Comparison between Adult and Fetal Hemoglobin



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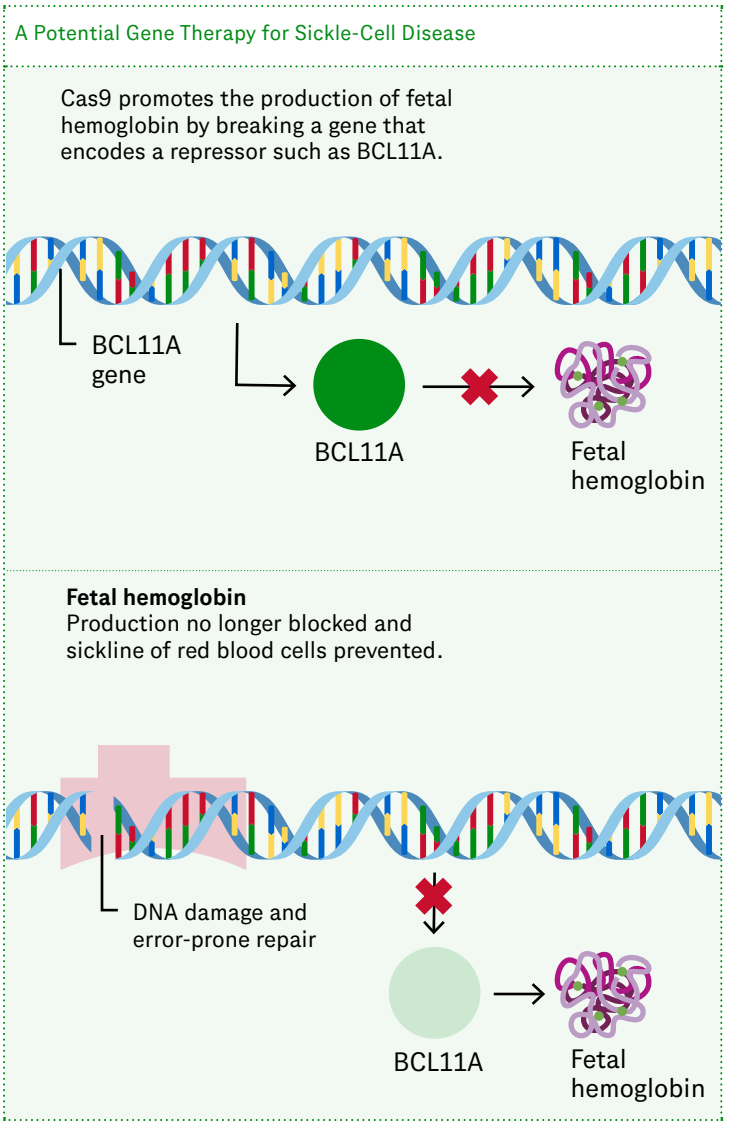
Using CRISPR-Cas9 to Combat Disease

Directions

Follow the steps on the subsequent pages to understand how CRISPR-Cas9 could be used to disable a repressor, allowing for fetal hemoglobin to be transcribed in adults as a potential therapy for sickle cell disease, as demonstrated by the cartoon on the right.

1. Go to NCBI to view the *Crystal structure of BCL11A* bound to γ-globin.

2. Click the *3D view* in the bottom left of the window to be able to move the molecules around. Note how the protein (repressor) is represented by pink alpha helices and beta pleated sheets, while the γ-globin DNA is shown in green and gold.



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Transcribing and Translating the BCL11A Repressor

Directions
Below is a sequence of DNA from the BCL11A repressor.
Follow the steps to visualize its normal folding pattern.

1. Cut along the solid lines and tape or paste the DNA strand by joining ends together (A tapes to A, B tapes to B, etc.).

2. Using the *mRNA codon chart*, transcribe and translate the underlined DNA sequences below (where AA stands for amino acid).

Sequence A

DNA	Ø	GTCT**GCT**TCCATCCAGACTCCTGACGTT**CTC**GTTCGCAGGGACGTCACGTCCGCACTTGA**TTT**TGCAGCT	A
mRNA		____ ____ ____	
AA		____ 1 ____ 4 ____ 3	
DNA	A	CAGGTTTTCTTTTGCCATTTTTTTCATCTCTCTCTCTCTCTCTCCCT**CAT**TCTCTCTTCTCTCTCTCTCC	B
mRNA		____	
AA		____ 5	
DNA	B	C**TAC**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTTAAAAAAAAAGC**ATA**GACGGCTCTCCCACAATTCAT	C
mRNA		____ ____	
AA		____ 6 ____ 2	
DNA	C	**CTG**CCCTGCGCCATCTTTGTATTATTTCTAATTTATTTTGGATGTCAAAAGGCACTGA**AAA**AGATATTTT	D
mRNA		____ ____	
AA		____ 3 ____ 6	
DNA	D	CTCTGGAGTCTCCT**GAT**TTCTAACCCGGCTCTCCCGATGTGAA**GTT**AGCCGTCGTCCGCCCGCCGCCGCC	E
mRNA		____ ____	
AA		____ 5 ____ 2	
DNA	E	GCCGCCGCCGCCG**GTT**CCCGCCCCGCAGCCCACCATGTCTCGCCGCA**GTG**AAGGCAAACCCCAGCACTTA	Ø
mRNA		____ ____	
AA		____ 1 ____ 4	

Continues next page >

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Transcribing and Translating the BCL11A Repressor

Continued

3. With your translated amino acid sequence, model protein folding by taping each numbered amino acid together. For example, amino acid 1 with amino acid 1 and so on. This structure represents the complicated, three dimensional structure of the functional repressor protein.

4. Recall that amino acids have different characteristics. Some are polar, some are non-polar, and others have opposite charges that are attracted to each other. In the table below, explain why each numbered amino acid will form a bond with each other. Refer to Sequence A above for the amino acid numbers.

Amino Acid #	Amino Acid Names (abbreviated)	Polar, Non-Polar, or Charged	In general, why do these form bonds the way they do?
1			
2			
3			
4			
5			
6			

Continues next page >

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Transcribing and Translating the BCL11A Repressor

Continued

5. The DNA sequence below is the same as *Sequence A,* representing the BCL11A repressor. We will use this to model how CRISPR-Cas9 cuts. Cut out and tape A to A as you did the previous sequence. Do not tape the amino acids together. Highlight the three-nucleotide-long *PAM* sequences (AGG, CGG, TGG, GGG).

   Note: When reading this piece of DNA, imagine you are flowing down a river as you read from left to right. When discussing positions on the DNA, you can say "that sequence is upstream" or "that site is downstream" from a position of interest.

Sequence B

DNA	Ø	GTCT**GCT**TCCATCCAGACTCCTGACGTT**CTC**GTTCGCAGGGACGTCACGTCCGCACTTGA**TTT**TGCAGCT	A
mRNA			
AA			
DNA	A	CAGGTTTTCTTTTGCCATTTTTTTCATCTCTCTCTCTCTCTCTCCCT**CAT**TCTCTCTTCTCTCTCTCTCC	B
mRNA			
AA			
DNA	B	C**TAC**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTTAAAAAAAAAGC**ATA**GACGGCTCTCCCACAATTCAT	C
mRNA			
AA			
DNA	C	**CTG**CCCTGCGCCATCTTTGTATTATTTCTAATTTATTTTGGATGTCAAAAGGCACTGA**AAA**AGATATTTT	D
mRNA			
AA			
DNA	D	CTCTGGAGTCTCCT**GAT**TTCTAACCCGGCTCTCCCGATGTGAA**GTT**AGCCGTCGTCCGCCCGCCGCCGCC	E
mRNA			
AA			
DNA	E	GCCGCCGCCGCCG**GTT**CCCGCCCCGCAGCCCACCATGTCTCGCCGCA**GTG**AAGGCAAACCCCAGCACTTA	Ø
mRNA			
AA			

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Transcribing and Translating the BCL11A Repressor

Continued

6. Now we will use Sequence B to model how CRISPR-Cas9 can change the function of a protein by cutting the DNA strand. Remember, the guide you will give to Cas9 needs to be an RNA guide. Identify the DNA sequence you will target and transcribe your target to RNA below.

   For example:

Target	A A C G G T A C G A C G A
gRNA	U U G C C A U G C U G C U

   Your Turn: (make sure your target sequence is somewhere that will affect the protein folding and near an NGG site).

Target	
gRNA	

7. Write the gRNA sequence you developed above into the *CRISPR-Cas9 Model.* Follow the directions on that page to model how CRISPR-Cas9 scans for matching target sites.

8. Recall from previous activities that the cell attempts to repair DNA that has been cut. However, in the attempt to repair, random nucleotides are introduced.

   For the purpose of this activity, we will assume that the random mutation introduced is ATT.

   Without transcribing and translating this shifted DNA sequence with the added ATT, predict how this insertion would impact the structure and folding of the modified protein.

   a. What does ATT code for?

   b. How will this affect the overall protein?

   c. How will this affect someone that has sickle cell disease?

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Barriers to CRISPR-Cas9 Therapies Capture Sheet

Directions
Read the following, then answer the questions on the next page about approaches to overcoming barriers to treatments.

According to the CDC, SCD affects approximately 100,000 Americans. It has a higher percentage in those whose ancestors came from sub-Saharan Africa, South America, the Caribbean, Central America, Saudi Arabia, India, and Mediterranean countries, such as Turkey, Greece, and Italy. SCD affects 1 out of 365 Black or African-American births in the United States. This is a significant number of people who need access to treatments for SCD. However, there are significant barriers to gene therapies. For example, they generally cost around $10,000 per month. Read more about the economic barriers to gene therapies in the article *Paying for CRISPR Cures: The Economics of Genetic Therapies* from Innovative Genomics Institute. Also, the number of SCD treatment centers in the United States is far fewer than those available for other diseases, such as hemophilia (a blood clotting disease that affects 20,000 people in the United States). The centers that do exist are poorly coordinated with primary doctors and are underutilized. Researchers concerned with and advocating for underrepresented groups participating in clinical trials for new therapies, such as CRISPR, have found that empathy and humility, along with understanding the cultural and historical factors at play, are key to patient involvement, awareness of, and access to new therapies.

Almost 25 years ago, the National Institutes of Health (NIH) issued a policy requiring that all NIH-funded clinical research include women and minorities, but there are still reports of failure to enroll minorities in clinical trials. Ineffective communication, lack of trust in research, and practical factors, such as time commitments and awareness of available treatments and clinical trials, all play a role in recruiting minority patients.

Researchers have found that fostering cultural humility can make a difference. Cultural humility is the commitment to self-evaluation and valuing unique and diverse populations and their characteristics. It requires openness, self-awareness, and being egoless. When researchers consider the culture and beliefs in the community and when they develop empathy for and engage in the community, it increases community knowledge and awareness of treatments. Recruitment for participation in new treatments need to be tailored to the community and communicated in a way that engages with the culture of the group.

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Barriers to CRISPR-Cas9 Therapies Capture Sheet

Continued

1. Why do you think it is important to include underrepresented groups in clinical research?

2. What role does cultural humility play in helping patients with SCD gain access to new treatments?

3. Some pharmaceutical companies understand the barrier to treatments are complicated. They provide grant funding to help patients with SCD gain access to treatments. A pharmaceutical company provided $500,000 to be used within the next year and if spent appropriately, the funding would continue for another year. If you were advocating for the funds to be spent appropriately, what are a few key factors you would include in your grant proposal (request for funds) to address the inequities people with SCD face in gaining access to high-quality, continuous healthcare?

   a. How would you ensure this money is used appropriately to maximize the benefit to underserved communities?

   b. How would you ensure cultural humility played a role in how the funds were spent?

   c. How could the money be spent to improve communication between clinics and patients with sickle cell disease?

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Codon Chart

Description

This tool is called a codon chart. It is used to decipher DNA into the amino acids that will be produced. You will use this chart to translate sequence A in the *Transcribing and Translating the BCL11A Repressor.*

Directions

Locate the first letter of your mRNA codon in the vertical column 1, the second in the horizontal column 2 and the third in the vertical column 3. Use the abbreviations below to determine which amino acid your codon codes for.

1	2				3
	U	**C**	**A**	**G**	
U	UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA, UAG: stop	UGU, UGC: Cys UGA: stop UGG: Trp	**U C A G**
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Pro	**U C A G**
A	AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg	**U C A G**
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	**U C A G**

Key

Ala = Alanine (A)
Arg = Arginine (R)
Asn = Asparagine (N)
Asp = Aspartate (D)
Cys = Cysteine (C)
Gln = Glutamine (Q)
Glu = Glutamate (E)
Gly = Glycine (G)
His = Histidine (H)
Ile = Isoleucine (I)
Leu = Leucine (L)
Lys = Lysine (K)
Met = Methionine (M)
Phe = Phenylalanine (F)
Pro = Proline (P)
Ser = Serine (S)
Thr = Threonine (T)
Trp = Tryptophan (W)
Tyr = Tyrosine (Y)
Val = Valine (V)

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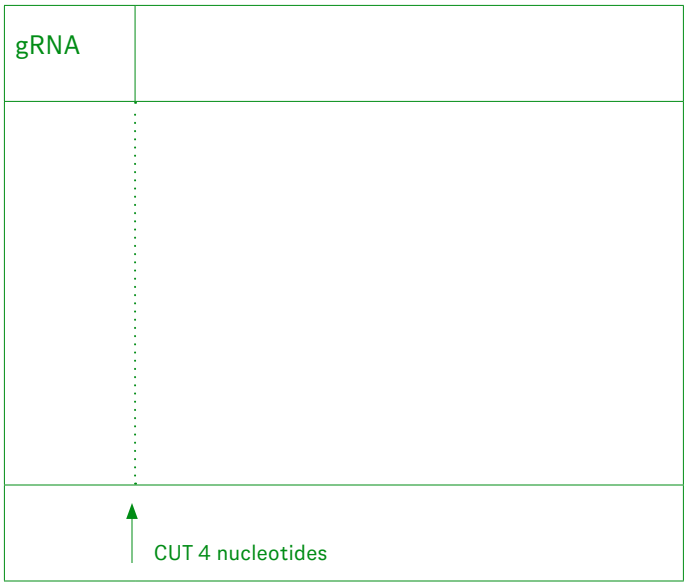
CRISPER-Cas9 Model

Directions

Write the gRNA sequence you developed on the Using CRISPR-Cas9 to Combat Disease Capture Sheet into the left column. Then cut the dotted line such that there is a pocket in the CRISPR-Cas9 Model. Pass Sequence B through the CRISPR-Cas9 Model to scan for the PAM sequence and the matching target to your gRNA. Cut Sequence B as CRISPR-Cas9 would.



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Microbe Phage Scenario Articles

Directions
*Select one of the following four scenarios to answer questions in your **Toolkit**.*

Scenario	1
Title	Bacteriophage in Food Fermentation
Source	*Bacteriophages in food fermentations: new frontiers in a continuous arms race*

Bacteriophages are the most abundant viruses on the planet, found universally in natural and man-made environments where bacteria thrive. Bacteriophages are specific to the subset of cells they target, eventually causing the cells they infect to lyse, or burst, releasing hundreds of new viral particles that can infect susceptible neighboring cells. Bacteriophages, which are also referred to as phages, are continuously functioning in a predator-prey relationship with other phages. They have the capability to evolve rapidly as a strategy to survive and persist in this evolutionary battle.

Bacteria are commonly used in industrial-scale food fermentations by inoculating fermentable substrates with selected bacteria to create a product with desired properties and functionalities. The portion of bacteria used to inoculate the fermentable substrate is called the starter culture. Starter cultures are typically composed of bacteria that were isolated from endogenous flora of the food. They are either well characterized (defined) from a microbiological and genomic perspective or unknown (mixed) with no known adverse effects on the product. Common bacterial strings used as starter cultures are lactic acid bacteria (LAB), which includes *Lactococcus lactis*, *Streptococcus thermophilus*, several *Lactobacillus spp.*, and *Leuconostoc spp.* (spp. is an abbreviation that indicates "several species"). Because industrial food fermentations are performed in large vats and under non-sterile conditions, they are susceptible to phage colonization causing fermentation failures. The most problematic situations impact the product quality rather than completely derail fermentation. These are extremely important because variations in the product taste and texture impact consumer trends and a product's reputation. Although many control measures have been investigated to eradicate all phages in food industry settings, no single method has proven to be effective. Many strategies have been developed to control phage populations, but it is nearly impossible to eliminate phages completely. Successful food fermentation factories have systematic preventative approaches to phage contamination, referred to as PACCP or phage analysis and critical control point. In order to systematically prevent phage contamination, all sources must first be carefully identified to implement measures to limit all possible phage introduction mechanisms. This assumes adequate phage detection methods and proper identification of which phages or phage groups are contaminating or evolving during the fermentation process. As such, the bacterial strains used in fermentation must be carefully considered. If unique strains are used, they should be highly refractory to phage infection. In many cases, multiple strains are used to produce a unique food product. It is mandatory to use strains with different phage sensitivity profiles to avoid complete lysis of the culture causing a failed fermentation. Phage resistance phenotypes of highly valuable bacterial strains can be improved by naturally modifying or adding genes coding for antiphage systems.

Phage resistance mechanisms are numerous and can be selected for target specific phages or groups. Naturally phage-resistant derivatives can be selected via exposure of wild-type phage-sensitive strains to specific phages. These spontaneous phage-resistant mutants are called bacteriophage insensitive mutants (BIMs) and are considered non-GMOs because they are produced through natural evolution. Numerous genetic engineering tools can also be used to develop phage-resistant strains without affecting their genetic properties. However the development of improved GMO strains is not preferred to engineering phage resistance.

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Microbe Phage Scenario Articles

Continued

Phages can contaminate fermentation cultures in several ways. Identifying the sources or reservoirs of phages within a factory is crucial to avoid introducing and disseminating new phages or a large quantity of viruses able to disrupt bacterial growth. Raw or heat-treated fermentation substrates and ingredients may contain phages. These components are not sterile and often cannot be sterilized, nor can the phage be completely eliminated without modifying their intrinsic and functional properties. Treatments, such as thermal exposure, high pressure, and ionizing radiation, can limit the phage load of raw materials without significantly modifying the food source. Some treatments are more effective at eliminating some phages, but may be ineffective against others. As such, appropriate measures must be selected on a case-by-case basis that considers ecofriendliness, the food itself, volume to be processed, and the cost of the system. Equipment and surfaces can have different levels of phage contamination in an industrial facility as well. Sanitizers and biocides (i.e., bleach) can be used to limit the ability to reduce the phage population. Air can also transport phage contamination through equipment producing aerosols. Adequate ventilation system and airflow control is of great importance to limit the phage contamination caused by aerosols.

Starter cultures may contain some prophages that can be activated through environmental stresses. Prophages represent 10% to 20% of genome content for some bacteria. When excised or cut, occasional temperate phages may lose their lysogenic gene cluster and become virulent. Conversely, prophages can benefit the bacteria. Prophages may confer resistance and enhance food properties such as flavor development through releasing enzymes.

Phage detection methods are necessary to identify phage contamination and phage dynamics. These methods must be sensitive to detect low levels of phages, easy to apply appropriate control measures, applicable with food matrices, easily performed by staff, and affordable. The traditional phage plaque assay is used as the standard microbiological method to detect phages which requires few materials to execute. However, this method has some drawbacks, including long incubation times, and some phages may not form plaques and cannot be analyzed, and results are not easily reproduced between different laboratories. Phages can be observed using transmission electron microscopy as well as with fluorescent compounds. As DNA-based methods are becoming publicly available at lower cost, new methods have been developed to detect phages in food matrices. These methods are more useful in phage classification, rather than screening and detection. An example is restriction fragment length polymorphism, which is used to discriminate between closely related phages. This technique is sensitive, fast, and discriminative, but because no phage gene is shared by all phages, this technique is limited because random primers will not necessarily amplify phage DNA. Another common technique is multiplex PCR, which is extremely useful to separate phages in the principal phage groups, but cannot discriminate fine differences between members of a specific group.

Monitoring phages during fermentation is a prime consideration, and classifying them in phage-related groups can aid fast adaptation of control strategies. Although phage classification was historically based on electron microscopy, genome sequencing has revealed critical information on phage biology, evolution, and classification. Phage genomes are highly compact with few noncoding regions. They are composed of modules of genes organized in clusters expressed in a specific order to achieve efficient phage multiplication and the completion of the lytic cycle. By comparing phage genomes, it is possible to study their evolution within manufacturing facilities. The growing database of sequencing data highlights the diversity of phages, but also has led to the identification of core phage genome elements encoding structural proteins.

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Microbe Phage Scenario Articles

Continued

Many microbiological strategies can be employed to control phage propagation. If suitable sets of bacterial strains are available, they can be rotated to avoid the build-up of specific phage populations within a factory. It is also possible to modify a bacterial strain through natural and genetically engineered methods into a phage-resistant derivative. Bacteria have several strategies to defend themselves against phage attacks through inhibition of phage adsorption, inhibition of phage DNA ejection, and cleavage of phage nucleic acids. With the growing number of complete phage and prophage genomes available in public databases, nucleic acid sequences can be used to identify and target essential phage replication steps and can inhibit their multiplication cycle.

Furthermore, regulator sequences, such as phage promoters, can be used to trigger bacterial suicide when cloned in front of a toxic bacterial gene. When facing selective pressure or growth-limiting conditions, phages and bacteria coevolve. Knowing the bacteria-phage evolution process helps in selecting strategies to control phage populations and improve fermentation. Thus, understanding phage dynamics during fermentation is crucial to ensuring good strain succession and even to control bacterial spoilage.

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Microbe Phage Scenario Articles

Continued

Scenario	2
Title	Marine Viruses NIH Article
Source	*Marine viruses—major players in the global ecosystem*

Viruses are one of the largest reservoirs of unexplored genetic diversity on Earth. Nowhere is the importance of viruses more evident than the oceans, where millions of virus-like particles are present in every milliliter of water. Viruses play a major role in the nutrient and energy cycling of marine microorganisms and affect the structure of microbial communities. The abundance of viruses exceeds that of bacteria and archaea by roughly 15-fold, yet they represent only 5% of the prokaryotic biomass. Viruses have a range of effects on oceans by altering geochemical cycles to structuring populations and communities.

To study the distribution and abundance of viruses in the ocean, scientists use flow cytometry (FC) to analyze viral particles by fluorescent staining of nucleic acid bases. Flow cytometry allows scientists to characterize sub-populations of both viruses and host cells to be based on their fluorescence and scatter. Viruses with a lower fluorescence signal were most abundant in the Arctic Ocean biome where heterotrophic prokaryotes with high nucleic-acid content are abundant. In contrast, viruses with a higher fluorescence intensity and scatter are characteristic of the Phycodnaviridae family of viruses which infect eukaryotic phytoplankton. These viruses were most tightly coupled to the Chlorophyll-a concentration, an indicator of the presence of photosynthetic cells. This data highlights emergent properties of viral infections such that not every member of the prokaryotic community is equally affected by viral infection. In turn, these emergent properties of viral infection impact nutrient cycling and carbon fixation. Viruses have been shown to impact microbial diversity directly and indirectly, by selectively killing the competitively dominant taxa as well as introducing new genetic traits by horizontal gene transfer.

Viruses that infect invertebrates and vertebrates are well-studied because of their impact on commercially important species, but we know very little about the reservoirs, sources and sinks of viruses on organisms that are not commercially significant. In the marine aquaculture industry, viral diseases cause enormous losses in production and revenue. For example, penaeid shrimp are extensively studied, which led to the discovery of white spot syndrome virus (WSSV). Similarly commercially significant finfish have been studied and a wide range of viral families have been identified including rhabdoviruses, birnaviruses, nodaviruses, reoviruses, and herpesviruses. To better understand the molecular diversity within many families of viruses, scientists have relied on nucleic-acid technologies to identify where viruses occur and their modes of transmission. Some viruses have broad host ranges and appear to circulate between marine waters and freshwaters. Phylogenetic analyses provide strong evidence of transmission in fish stocks in North America as well as in Europe and Asia. Phylogenetic analyses rely upon similarities in their genomic DNA and allow scientists to identify common ancestors and evolutionary mechanisms. As such, scientists have identified that European freshwater viruses had a common marine ancestor approximately 50 years ago, and diverged from their North American marine and freshwater counterparts 500 years ago. However, it still remains unclear about viruses' natural reservoirs.

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Microbe Phage Scenario Articles

Continued

One technique that has been especially effective has been metagenomic approaches to catalogue marine virus communities. High-throughput pyrosequencing was used for metagenomic analysis including between 41 and 85 individual samples from the Arctic Ocean, the coastal waters of British Columbia, the Gulf of Mexico, and the Sargasso Sea. Of the approximately 1.8 million sequences obtained, more than 90% had no recognizable homology to previously reported sequences in the database GenBank.

Furthermore, BLAST (Basic Local Alignment Search Tool) homologue frequency to protein sequences within the GenBank non-redundant database is only approximately 30%. This highlights the lack of data in existing databases characterizing viral diversity. Metagenomic approaches enable scientists to capture the genetic richness of marine viral communities and characterize previously unknown viral genomes.

Interactions between viruses and the organisms they infect impact the genetic diversity and influence the composition of microbial communities. The composition of prokaryotic and viral communities at a specific location follows a steeply declining abundance-curve, which describes the relationship between K-selected and r-selected organisms. Viruses that are r-selected have large burst size, short generation times, and often kill their hosts compared to K-selected viruses which have longer generation times and coexist with their hosts for extended periods. Viruses with high growth and loss rates and rapid responses to environmental changes are generally r-selected. For viruses infecting prokaryotes, it is predicted that K-selected phages with small genomes and burst sizes will be more abundant. Viruses that infect photosynthetic and heterotrophic protists are expected to be virulent and have high reproductive rates, so as to take advantage of high growth rates and rapid responses to environmental changes. Viruses that infect large organisms, such as crustacean zooplankton, fish and mammals, will be K-selected and are expected to have a lifecycle that depends on non-virulent and close association with that of its host. Ultimately, an organism will be affected by a range of viruses along the r-K selection continuum.

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Microbe Phage Scenario Articles

Continued

Scenario	3
Title	Bacteriophage and Human/Animal Health NIH Article
Source	*Bacteriophages: an underestimated role in human and animal health?*

The development of metagenomics analyses in parallel to other scientific approaches have allowed scientists to characterize the high level of diversity of the viral fractions of ecosystems in what is called a "virome." The viral fraction is composed of viruses of bacteria, also known as bacteriophages, which until recently have been largely overlooked and uncharacterized. The diversity of microbial communities living in symbiosis with the human body has been described in the digestive tract, human saliva, the respiratory tract, and skin. The gut microbiota is a complex microbial ecosystem, comprising more than 500 species living within their host. Changes in the abundance of bacterial composition have been linked with several human disorders and disease, including Panton-Valentine, cholera, Shiga- and diphtheria toxins.

Phages belong to two categories, virulent and temperate. Virulent phage life cycles replicate within their host causing them to burst or lyse, while temperate phages can alternate between lysing their host or establishing a symbiotic, or equally benefitting both host and phage, relationship known as a lysogeny state. Upon bacterial infection, the fraction of temperate phages that enter into lysogeny varies and is generally low compared to the fraction that go into lysis. In the condition of lysogeny, the phage is named "prophage." Known signals of prophage induction are DNA damage, temperature, and oxidative stress. Some prophages disrupt bacterial genes, which are restored upon phage excision leading to a phenotypic change. Poly-lysogenic strains can exhibit a range of phenotypes depending on which prophage is expressed. Thus poly-lysogens can exist in multiple states, including production of background levels of virions, excision of a prophage and appearance of a new phenotype by restoration of the gene that was interrupted by the prophage, and adsorption of virions at the bacterial surface and use as virulence factors.

Virome studies allow us to characterize all phage genomic diversity, which is far greater than bacterial genomic diversity. Phage genomic diversity is huge in the gut microbiota and the functional analysis of viromes is both understudied and difficult to interpret.

The number of phage particles is equally as important as phage diversity, and provides insight into the phage roles in ecosystems. "Virus like particles" (VLP) counts are based on fluorescent dye labeling of samples followed by microscopic analysis. In the digestive tract, bacteria density increases along the digestive tract from the almost sterile stomach environment to the colon and in feces, where VLP estimates appear to be much lower.

Phage dynamics are expected to be radically different if the environment is structured rather than homogenous as in aqueous solutions. Validated in a series of experiments, phages were shown to multiply more readily on a semi-solid agar compared to liquid medium. The gut is structured into compartments that can be distinguished lengthwise and crosswise. Within each segment, there are three layers: the mucus layer, the lumen, and the food particles. In addition to the spatial compartments, water content, pH, and oxygen concentration vary greatly according to the gut segment, in turn affecting the replication of phages and highlighting why they tend to replicate better in the upper tract.

Phages can impact a bacterial ecosystem by predation and multiplication on susceptible bacteria. Temperate phages can also lyse their previously lysogenic host upon induction, but can also carry genes that modify bacterial phenotypes.

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Microbe Phage Scenario Articles

Continued

Phages rely on chance encounters through random collision and their ability to infect and reproduce is determined by host density. In a concept known as “kill the winner” phages kill only the dominant member of the ecosystem if the population of bacterial prey is high. However, this model is only rarely observed and difficult to replicate under laboratory conditions. Bacteria can use their prophages as biological weapons against their main competitors; related bacteria that occupy the same ecological niche. Rare, spontaneous prophage induction in a small fraction of the lysogenic population can trigger an epidemic among susceptible bacterial competitors, which become factories for producing more phage. By killing the competitor, prophages can indirectly benefit their bacterial host. Prophages can also be detrimental as their induction results in host lysis.

Even if spontaneous induction is a rare event, a variety of environmental factors can change induction from a rare event to a deterministic process. In the gut, several inducing agents can trigger prophage induction such as antibiotics like quinolones. Oxidative stress is another potent inducer of phages in the gut.

Temperate changes can also propagate by establishing lysogeny into the host they infect, ratherthan lysing it.

Recently, several metagenomic studies have identified the presence of antibiotic resistance genes in the virome fraction. These genes have been associated with transposons or insertion sequences. Phages have the remarkable capacity to mutate and recombine at rates orders of magnitude higher than bacteria. Temperate phages efficiently exchange sequences with defective phage remnants present in the cell in which they multiply. New phages are therefore constantly emerging.

Phages play an important role in all bacterial ecosystems, but their precise impact remains not fully understood. Present as the major component in most ecosystems, phages appear to be less numerous in the intestine where fecal VLP counts are lower than bacterial counts. The gut environment also differs from most other ecosystems containing a majority of temperate phages, resulting in spontaneous induction of prophages integrated into the bacterial genomes.

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Microbe Phage Scenario Articles

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Scenario	4
Title	Bacteriophage and COVID Article Published in Phage Journal
Source	*Bacteriophages Could Be a Potential Game Changer in the Trajectory of Coronavirus Disease (COVID-19)*

The coronavirus pandemic is caused by the emergence of a new virus, SARS-CoV-2 or "Severe Acute Respiratory Syndrome CoronaVirus 2." The SARS-CoV-2 has spread at an unprecedented rate, affecting the global health and economy. The use of bacteriophages serves as a potential tool to decrease mortality among patients infected by the virus.

SARS-CoV-2 is an airborne virus affecting the respiratory system. The indirect cause of death in Covid-19 patients may be attributed to the difference between the innate and adaptive immune response. The adaptive immune response is much slower than the innate or natural immune response to target a new pathogen. As such, after initial infection the innate immune response attacks the new pathogen often being too aggressive when faced with high viral load, which can damage other systems. As the virus replicates within the host, the innate immune system secretes fluid and inflammatory cells into the lungs causing them to fill with fluid and reducing the body's ability to exchange gases. The debris of dying and virally infected respiratory cells become a substrate for bacteria growth and give rise to bacterial infection such as pneumonia. This process accelerates as the disease progresses, adding too much inflammatory fluid to the lungs and inhibiting gas exchange, which can cause sepsis and death. Recent studies have highlighted the differences in disease progression between younger and older patients as it relates to the delay or failure to produce antibodies. Older patients are more susceptible to immunosenescence, or the impairment of immune functions, which contributes to the higher rate of mortality among older Covid-19 patients.

The co-occurrence of viruses and bacteria has been well-documented in viruses in what is considered "succession" by ecologists and "secondary infections" by medical professionals. *Staphylococcus aureus*, *Staphylococcus pneumoniae* (*pneumococcus*), *Aerococcus viridans*, *Haemophilius influenza*, and *Moraxella catarrhalis* are bacteria typically found in influenza patients, as well as other respiratory diseases, which can turn into pathogens causing infection. *Acinetobacter baumannii* and *Klebsiella pneumoniae* have been found in Covid-19 patients with a higher occurrence of sepsis and secondary infection in non-survivors. The high mortality rate of Covid-19 can be partially attributed to bacterial infection of the respiratory system, but it remains an ongoing challenge to document all cases seen in clinics.

A recent study in Wuhan shows that at least 50% of patients dying developed secondary infections. It is plausible that bacterial infections begin to colonize before acute respiratory syndrome is developed. However, the interplay between the time taken for the human body to develop antiviral antibodies and the role of bacteria in the death of older individuals is not well known for Covid-19.

A promising strategy to reduce bacterial growth in the respiratory system of patients and lower Covid-19's mortality rate has been with the use of bacteriophages. Bacteriophages selectively attack bacterial species and are otherwise harmless to animal cells. Bacteriophages attach themselves to susceptible bacteria, exclusively infecting the host bacterial cells, and hijack the bacterium's biochemical machinery to produce multiple copies of itself. The infected bacterium then undergoes lysis, releasing new copies of bacteriophage to infect other bacteria of the same species. While bacteriophages are a promising strategy to treat bacterial infections, antibiotics have been the predominant method to treat bacterial infections. Antibiotics are used for general purpose because they are usually fast-acting, efficient, and relatively cheap to manufacture. However, antibiotics are not as specific as bacteriophages and target beneficial bacteria in addition to harmful ones.

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The overuse of antibiotics also gives rise to antibiotic-resistant bacteria that become even harder to treat. Around 70% of hospitalized Covid-19 patients receive antibiotics in their treatment, which increases the likelihood of antibiotic-resistant strains and the need for alternative strategies to fight bacterial infection. Bacteriophage treatments would be far less susceptible to the development of resistance, as the bacteriophage can adapt to overcome any resistance that the bacteria it targets may develop.

Bacteriophages have been successfully used as a tool at the molecular level with phage display. Bacteriophages have the potential to quickly produce recombinant antibodies and have been successfully applied to MERS-CoV, another severe respiratory pathogen. There are two main ways that bacteriophages could be used to decrease the mortality rate of the Covid-19 pandemic. They can be used to decrease the population of bacteria in a patient's respiratory system and/or bacteriophage display techniques can be used to efficiently manufacture synthetic antibodies against SARS-CoV-2. Bacteriophage treatment has been shown to cure pneumonia by reducing lung bacterial burdens and improving survival of antibiotic resistant *S. aureus* infected animals in the context of ventilator-associated pneumonia. Selecting bacteriophages and optimal target bacteria could be quickly engineered by microbiologists as the bacterial species that commonly cause respiratory problems are well known.

A risk of this technique is bacteria developing resistance to the bacteriophage, yet this would be much less severe than antibiotic-resistant bacteria. In bacteriophage therapy in the pneumonia system, the rapid lysis of bacteria by bacteriophages does not increase the innate inflammatory response. This is promising because it shows that there is a positive effect on the patient's immune system and does not contribute to disease progression.

While the response to antibiotics may be slower or smaller than expected, bacteriophages decrease the population growth rate of bacteria effectively. Intensive use of antibiotics targeting Covid-19 in clinics leads to bacterial resistance in the hospitals, emphasizing the need for alternative therapy to reduce risk of resistance. If bacteriophage treatment can be developed, it is a practical solution with the potential to be produced quickly and cheaply.