Last Updated: 09/07/25

How Science Works

Student Workbook

10th & 11th Grade - CRISPR

Scientist Name: _____

Teacher Name: _____

SciTrek Mentor Name: _____

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Welcome to SciTrek!

Hey there! Welcome to SciTrek—we're so excited to have you join us! This workbook is your guide to all the cool science activities and experiments you'll be doing throughout the program. You'll get to dive into some amazing concepts, work in teams, and learn from SciTrek Mentors and Leads who are here to help you explore the world of science.

Here's what to expect:

- **Day 1:** You'll kick off the program with 2-4 SciTrek Leads who will guide you through the start of your adventure.
- **Day 2:** Get ready to roll up your sleeves! You'll work with a group of 5-6 SciTrek Mentors, diving into hands-on science activities and experiments in small teams.
- **Day 3:** It's your time to shine! You'll complete your work on the previous day's experiments with your small group team and 2-4 SciTrek Leads.
- **Day 4:** The SciTrek Mentors are back! You'll continue working on exciting experiments in small groups, with plenty of support from the mentors.
- **Day 5:** Time to wrap it all up! With SciTrek Leads, you'll close out your time in the program and reflect on everything you've discovered.

Remember, your teacher and the SciTrek team are here to help you every step of the way, so don't be afraid to ask questions or share your ideas. This is your chance to explore, experiment, and have fun with science! Let's make this an unforgettable experience!

What You'll Learn in the CRISPR Module

In this module, you're going to dive into awesome science concepts - some may be familiar, and some may seem brand new! - all while meeting the important goals that scientists follow every day. Here's what you'll be able to do by the end of your time in the program:

- **Micropipetting:** You will demonstrate accurate use of micropipettes to measure and transfer small volumes using correct technique.
- **Bacteria Plating:** You'll learn and practice successful techniques for live bacteria plating.
- **Gene Editing:** You'll formulate a hypothesis and demonstrate your knowledge of gene editing and CRISPR/Cas9 through hands-on experimentation.
- **Variable Testing:** You'll test variables in experiments and recognize the importance of changing one variable at a time to make strong conclusions in experimental design.
- **Consideration of Ethics:** You'll engage in thoughtful discussion on the ethical concerns surrounding gene modification technology.
- **Spotting Mistakes:** You'll learn how to identify errors in experiments and understand the limits of your measurements.
- **Hypothesis Testing:** You'll develop your own ideas, test them out, and see if your predictions were right.
- **Data Analysis and Presentation:** You'll learn how to analyze your results, create graphs, and share what you've discovered through a slideshow presentation.

Get ready to explore, experiment, and present like a real scientist!

Important Vocabulary

Agar – A nutrient-rich, jelly-like substance obtained from red seaweed and often used for growing bacteria.

Bacterial Growth Rate – The speed at which bacteria multiply and form visible colonies on the plate.

Colony – A visible cluster of bacteria that arises from a single bacterial cell in a petri dish or on a lab plate.

Contamination – The unintended introduction of foreign microorganisms into a sample, which can skew results and reduce accuracy.

CRISPR/Cas9 - Genome editing technology that allows for precise and targeted changes to genomic DNA in living organisms. CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats." Cas9 stands for "CRISPR-associated protein 9."

Culture – A population of bacteria or other microorganisms grown under controlled conditions.

Designer Genes - Genetically modified genes that are selected solely on the basis of preference or style, and not a medical concern.

DNA - The "instruction manual" that tells each cell what to do, sometimes called "the code of life." DNA is made of four different repeating building blocks represented by the letters A, T, C, and G.

Gene - A piece of DNA that codes for a single protein. A gene is made up of a sequence of DNA letters.

Gene Editing - Purposefully eliminating, adding, or changing the letter sequence that makes up DNA. Since DNA letters control the traits of a gene, a scientist can change an organism's traits by editing its DNA.

Gene Knockout - A genetic engineering technique that inactivates, or deletes, a specific gene in an organism.

Guide RNA (gRNA) - A short RNA molecule that locates specific DNA sequences, and guides

Cas9 to its precise location for editing. gRNA is designed to match the specific DNA sequence that needs to be edited.

Hypothesis - A hypothesis is a testable guess or prediction about what will happen based on what you know.

Incubation – The process of maintaining cultures at a specific temperature to promote bacterial growth.

Inoculation – The process of introducing microorganisms, such as bacteria, into a new medium or environment for growth.

IPTG - A reagent that causes the activation of the CRISPR proteins that make the cuts in the LacZ gene, which is located past the PAM site.

Microliter (µL) - A microliter is a unit of volume in the metric system equal to one-millionth of a liter ($1\ \mu L = 10^{-6}\ L = 0.001\ mL$). It is commonly used in molecular biology, microbiology, and chemistry for measuring small liquid volumes.

Micropipette – A laboratory tool used to measure and transfer very small volumes of liquid, typically expressed in microliters (µL).

Milliliter (mL) - A milliliter is a unit of volume in the metric system equal to one-thousandth of a liter ($1\ mL = 10^{-3}\ L = 1000\ \mu L$). It is frequently used in laboratory settings for measuring liquids in small, but not microscopic, quantities.

Non-Target Strand - The paired strand of foreign DNA cut by Cas9, whose sequence is not checked. A perfect match, its sequence matches the guide sequence.

Observation - information gathered using your 5 senses.

Opinion - belief that may or may not be based on facts.

Outliers - Data significantly different from other data in the same experiment.

PAM - PAM stands for "protospacer-adjacent motif," a short sequence of letters in the target DNA strand. Cas proteins only cut DNA with their specific PAM.

Petri Dish – A shallow, flat dish used to culture bacteria, typically made of glass or clear plastic.

Plasmid - A small, circular piece of DNA found in bacteria. It's separate from the bacteria's main DNA and can carry extra genes, like ones for antibiotic resistance. Scientists often use plasmids in genetic engineering to insert new genes into bacteria.

Quadrant Streaking – A technique used to isolate a pure bacterial culture by streaking a diluted sample across the surface of an agar plate.

Reagents - Substances introduced to a system in order to cause a chemical reaction.

RNA - Nucleic acid that carries genetic information from DNA to make proteins and regulate genes. RNA is made of four different repeating building blocks represented by the letters A, U, C, and G.

Selective Media – Media that contains substances that promote the growth of certain types of bacteria, while restricting the growth of others.

Sterilization – Free of live germs, bacteria, and any other microorganisms. Sterile materials in a lab are important in preventing contamination.

Target strand - The strand of foreign DNA that Cas proteins recognize using gRNA.

Volume – The amount of liquid measured or transferred, typically expressed in microliters (µL) or milliliters (mL).

Day 1

The Science of Small: A Guide to Micropipetting and Bacteria Plating

Objective

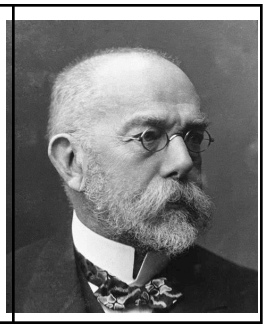
Today, we will focus on mastering your skills in the expert and accurate use of a micropipette, and explore other essential techniques for bacteria plating. You will explore a variety of "best practices" for bacteria plating such as how to accurately measure and transfer very small liquid volumes, how to avoid air bubbles, and even how to avoid contaminating or breaking your sample! Your new skills will prepare you for your gene transformation activities on Days 2&3. Ready for some cool experiments?

Introduction to SciTrek

What's the Plan?

- Meet the SciTrek Leads.
- Learn best practices for micropipetting - The function and most common uses of a micropipette, how to use micropipettes correctly, how to measure liquid volumes precisely, tips for improving your micropipetting skills, and common mistakes to avoid when micropipetting.
- Learn bacteria plating techniques – The purpose of bacteria plating, the three main methods for preparing and introducing bacterial cultures to new environments, tips for successful plating and avoiding contamination, and common mistakes to avoid when plating bacteria.

Robert Koch: The Father of Bacteriology and Disease Discovery
Robert Koch (1843–1910) was a German microbiologist known for identifying bacteria causing life-threatening diseases like tuberculosis, cholera, and anthrax. He developed a set of criteria to test whether or not a given microbe causes a disease, called "Koch's postulates." His work in bacteriology, including culturing techniques and staining methods, laid the foundation for modern microbiology. Koch won the Nobel Prize in 1905 for his discovery of



the tuberculosis bacteria.	

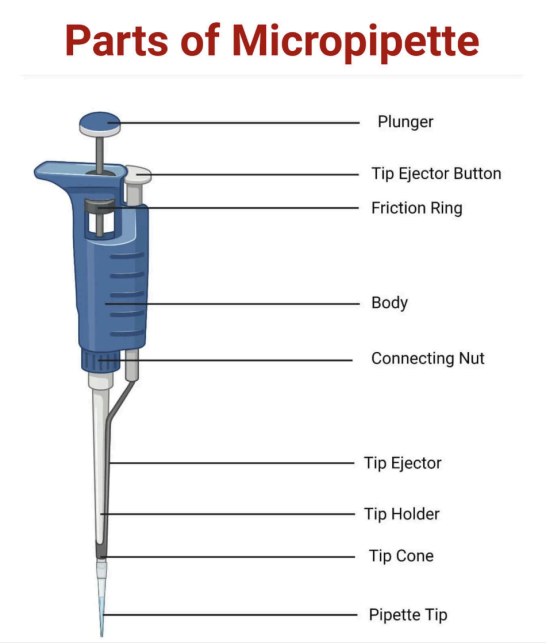
Introduction to Micropipetting

Key Content

1. What is a Micropipette?

- A micropipette is a laboratory tool used to measure and transfer very small volumes of liquid, typically expressed in microliters (µL) and ranging from 0.1 µL to 1000 µL (1 mL).
- Micropipettes are widely used in laboratories for tasks requiring high accuracy, such as DNA/RNA analysis.

2. Parts of a Micropipette



Tip Ejector: The mechanism on a micropipette that allows for the safe removal of disposable tips after use.

Disposable Tips: Single-use, sterile tips that attach to the micropipette for liquid transfer.

3. Types of Micropipettes

- **P1000**: Measures volumes from 100 to 1000 µL.
- **P200**: Measures volumes from 20 to 200 µL.
- **P20**: Measures volumes from 1 to 20 µL.

- **P10:** Measures volumes from 0.5 to 10 µL.

4. How to Use a Micropipette

1. **Set the Volume**: Adjust the volume by rotating the dial to the desired value.
2. **Attach the Pipette Tip**: Ensure the disposable tip is securely attached to avoid leakage.
3. **Extract the Liquid**: Press the plunger down to the first stop and hold, insert the tip into the liquid, and release the plunger slowly to draw the liquid up into the pipette.
4. **Dispense the Liquid**: Press the plunger down to the second stop to release all of the extracted liquid.

5. Tips for Accurate Micropipetting

- Always use a new, clean pipette tip for each sample to avoid contamination.
- Hold the micropipette vertically
- Use a consistent, smooth motion when pressing the plunger to avoid air bubbles.
- Change pipette tips between different reagents or samples to prevent cross-contamination.

6. Common Mistakes to Avoid

- **Air Bubbles**: Avoid creating air bubbles by gently and smoothly pressing and releasing the plunger. Extract and dispense slowly to prevent this.
- **Incorrect Plunger Pressure**: Applying too much pressure can lead to inaccurate volumes.
- **Incorrect Tip Attachment**: Always ensure the pipette tip is securely attached to avoid leakage.

Discuss with a classmate - What is a micropipette, and what is it used for? Write your definition below, including both a description of what a micropipette looks like, and how it could be used in a laboratory setting.

_____

_____

_____

Can you think of any experiments or tasks in the lab where a micropipette would be essential?

_____

_____

_____

Activity 1: Micropipette Reading Practice

Milliliters to Microliters Conversion Chart Example:

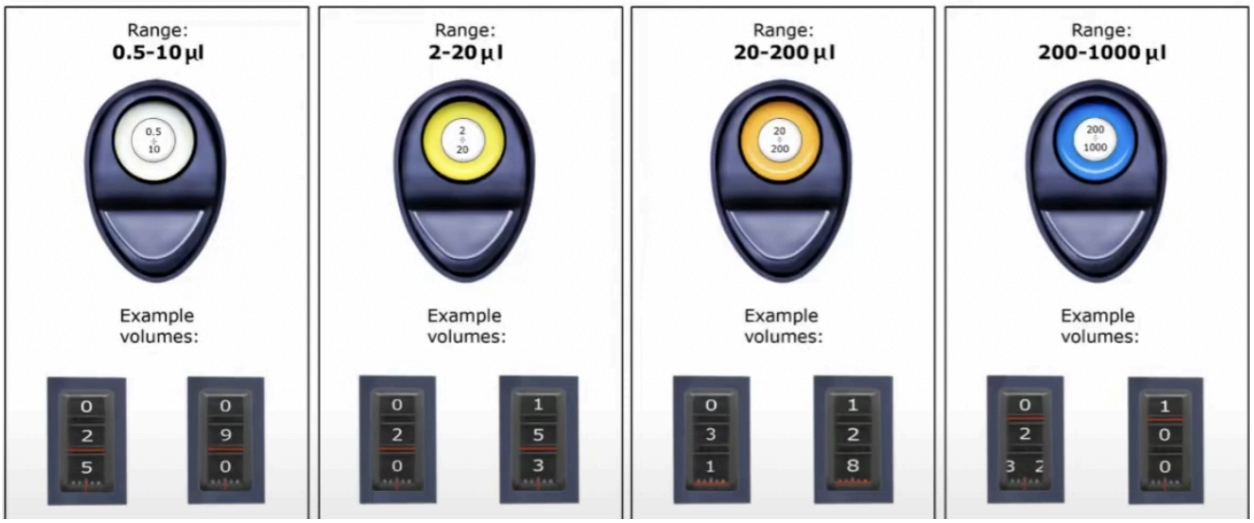
Milliliters to Microliters Conversion Chart
1 milliliter (mL) = 1000 microliters (µL)
0.1 mL = 100 µL
0.01 mL = 10 µL
0.001 mL = 1 µL

- Hint: To convert from mL to µL, multiply the number by 1000.
  Example: 0.15 mL × 1000 = 150 µL

  To convert from µL to mL, divide the number by 1000.
  Example: 500 µL ÷ 1000 = 0.5 mL

- Not all micropipettes display volume the same way! For example, some use 3 digits (Ward's Science brand), and others use 4 digits (VWR brand). Each digit represents a different place value, depending on the pipette type.

In the micropipette image below, select four values—each corresponding to a different micropipette type (based on their volume ranges). For each value, write down the displayed value and its actual volume in microliters (µL) in the data table below.



MICROPIPETTE TYPES DATA TABLE:

Pipette Type	Dial Display	Volume (µL)
P10		
P20		
P200		
P1000		

Key Questions:

What would the display look like when pipetting 200µL using the following micropipettes? Draw or describe the number shown in the display window for each.

P200 micropipette (Brand: Ward's science)

What Each Digit Represents	Display
Hundreds	
Tens	
Ones	

P1000 micropipette (Brand: VWR or Ward's science)

What Each Digit Represents	Display
Thousands	
Hundreds	
Tens	
Ones	

What would the display look like when pipetting 5µL using the following micropipettes? Draw or describe the number shown in the display window for each.

P10 micropipette (Brand: minipcr)

What Each Digit Represents	Display
Tens	
Ones	
Tents	

P20 micropipette (Brand: VWR)

What Each Digit Represents	Display
Tens	
Ones	
Tents	
Tenths	

Which micropipette would you use to accurately measure 0.025 mL?

(*Hint: Convert to microliters first.*)

_____

_____

True or False: You should use a P200 to pipette 0.005 mL.

Explain your answer.

Convert 0.150 milliliters (mL) to microliters (µL). Record your new value below.

(Hint: Multiply by 1000. Make sure to double-check your answer!)

If a P1000 micropipette is set to 650µL, what volume is it set to in milliliters (mL)?
(Hint: Multiply by 0.000001. Make sure to double-check your answer!)

If a P10 micropipette is set to .009µL, what volume is it set to in milliliters (mL)?

Activity 2: Micropipetting Activity - Color Mixing Challenge

Instructions:

- Wear protective gloves before beginning.
- Review your activity worksheet and examine the labels on the pre-marked test tubes. Each tube requires specific volumes and color combinations.

  Each group will receive:
  - 4 pre-filled source tubes (R, B, Y, G — Red, Blue, Yellow, Green)
  - 4 empty target tubes labeled A, B, C, and D.

- Work independently on the question below, using the conversion chart and pipette visuals from "Activity 1: Micropipette Reading Practice" for reference.

  Write the minimum and maximum volume you can pipette with each of the following pipettes:

Pipette	Volume Range (µL)
P10	_____ to _____
P20	_____ to _____
P200	_____ to _____
P1000	_____ to _____

- Identify which types of pipettes are needed based on the volume indicated.

<u>Target Tube Mixing Instructions:</u>
(Hint: Ensure all tubes receive the same total volume (250 µL) for better comparison of pipetting accuracy.)

Tube	Instructions	Expected Color
A	136 µL Red + 114 µL Yellow	Orange
B	250 µL Blue	Blue
C	59 µL Green + 7 µL Red + 184 µL Yellow	Olive green
D	125 µL Red + 125 µL Blue	Purple

E	Create your own color!	Try making your own color combination! Record the volumes used and name your new color.'

(Hint: Refer to the "Key Content" box on pages 9-10 for pipette types.)

- Begin by measuring and extracting the first colored water sample.
    - Choose the correct micropipette for each transfer.
    - Set the correct volume on the dial.
    - Replace the disposable pipette tip between each transfer to prevent contamination.
    - Add the correct color(s) to the correct target tube.

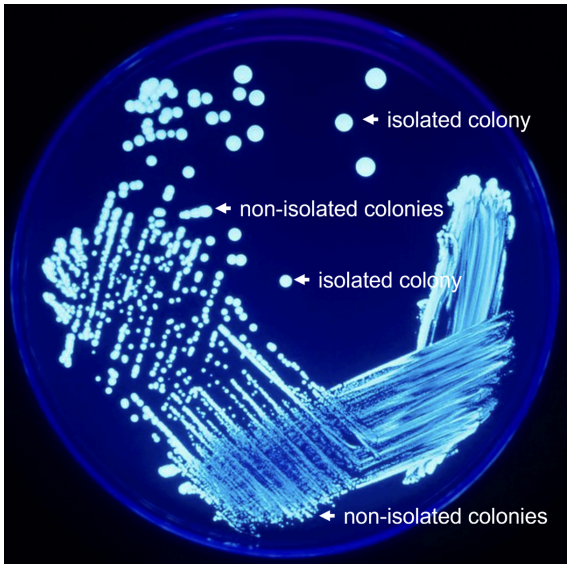
Goal: At the end, the color mixtures in tubes A, B, C, and D should visually match the expected results (e.g., orange for red + yellow, purple for red + blue).

(*Hint: Press and release the plunger slowly and smoothly to avoid air bubbles.*)

- As a group, discuss the questions below:
    - Were the volumes accurate? Were air bubbles avoided?
    - Did everyone follow best practices for changing tips?
    - What could your group do to improve your pipetting technique, in preparation for more advanced pipetting during gene editing on Days 2 and 3?

Activity 3: Colorful Cultures

In this activity, you will practice bacterial plating methods using food coloring as a substitute for bacterial cultures. Remember, while the colors do not represent real bacteria, each solution is meant to symbolize a different "strain." You will learn how to streak for isolation, and sharpen your new skills in pipetting accuracy.



Key Content

In order to keep bacteria samples contained, controlled, and clean for study, scientists will often place the samples onto a sterile (free of live germs, bacteria, and any other microorganisms) plate or dish. This is called **bacteria plating**.

- A **petri dish** is a shallow, flat dish used to culture bacteria, typically made of glass or clear plastic.
- A bacteria **culture** is a population of bacteria or other microorganisms grown under controlled conditions.
- The process of introducing microorganisms, such as bacterial strains, into a new medium or environment for growth is called **inoculation**.

One bacteria plating technique is called "**quadrant streaking**." Quadrant streaking involves creating a small, concentrated colony from a larger colony. This gradually results in isolated colonies which naturally group together by genetic sameness.

In this activity, you will be provided with a petri dish containing agar. **Agar** is a nutrient-rich, jelly-like substance obtained from red seaweed and is often used for growing bacteria.

Instructions:

1. **Label:** Write your name, date, and the "strains" (colors) you will be using on the bottom of your agar plate.
2. **Prepare:** Disinfect your work area and put on your protective gloves before you begin this activity. This is for your safety and the safety of those around you.
3. **Extract:** Using a micropipette set to **50µL**, extract your first sample of food coloring, which represents your "bacterial culture".
4. **Dispense:** Pipette a small spot of color onto the agar surface.
5. **First Streak:** Using an inoculating loop, dip the tip into the culture on your agar surface and drag the loop gently across one section of the plate in a zigzag motion.

   (Hint: Be very careful, as the agar may be damaged more easily than you think!)

6. **Second Streak:** Rotate the plate 90°, replace the loop/swab, and pull some color from the first streak into a new quadrant.
7. **Third & Fourth Streak:** Repeat until you've streaked all 4 quadrants.
8. **Close & Wait:** Close the petri dish and wait a few minutes as a group for the solution to dry.
9. **Final Placement:** Place the petri dish upside down. This step helps prevent condensation from warping your results.
10. **Label:** Label the plate on the bottom edge with the small group's information (table #, period #, name), so that your specific strain (color) growth can be tracked and observed over time.

Key Questions:

1. Compare the color of your first streak and your fourth streak. Do you notice any visible differences between these colonies? Describe.

_____

_____

2. Why is quadrant streaking important in this experiment? How could it help us isolate and analyze bacterial colonies when testing for successful CRISPR edits?

_____

Wrap-Up and Reflection

Recap:

- **Micropipettes:** There are many different kinds of micropipettes, which vary based on the volume of liquid that they can hold. Best practices for micropipetting include:
    - Using gentle and smooth pressure to avoid air bubbles.
    - Replacing tips to avoid contamination.
    - Maintaining a steady pressure to avoid inaccurate readings.
- **Bacteria Plating - Streak Technique:** One bacteria plating technique is called "streaking." Streaking involves creating a small, concentrated colony from a larger colony. This gradually results in isolated colonies which naturally group together by genetic sameness.

Preview for Day 2:

Get excited for tomorrow's session where we'll dig deeper into bacteria plating and begin the first half of our gene mutation transformation with your UCSB Mentors. See you there!

End of Day 1

Day 2

Introduction to CRISPR

Objective

Today, we will dive into the science behind gene modification and CRISPR/Cas9 technology. You will test your new knowledge on bacteria plating techniques as you begin your first steps on your gene transformation activity. Ready for some cool experiments?

Key Content

You have probably heard that certain traits that make up who we are - such as health predispositions, how we look, and even whether or not we are lactose-intolerant - are a result of our genetic makeup, or our genes. But what are genes, and does gene "editing" really work?

A **gene** is a piece of DNA that has the information to make another nucleic acid (RNA), which in most cases is then used by the cell to make a protein (translation). A gene is made up of a sequence of DNA letter bases. In the process of **gene editing,** a scientist purposefully eliminates, adds, or changes the letter sequence that makes up DNA. Since DNA letters control the traits of a gene, a scientist can change an organism's traits by editing its DNA.

CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats." **Cas9** stands for "CRISPR-associated protein 9."

CRISPR is a natural, bacterial "protective" system that guards against viruses. However, scientists now use this tool for gene editing! In nature, bacteria use CRISPR and the Cas9 protein to recognize and cut viral DNA. Researchers have adapted this system by designing guide RNAs to direct Cas9 to cut specific genes in other organisms. In both cases, Cas9 is the protein that makes the cut—whether it's defending bacteria or editing genes in the lab.

But *how* are scientists able to edit genes, when genes are part of the DNA already in a cell? **CRISPR/Cas9** is a highly effective gene-editing technology that allows researchers to edit parts of the genome by removing, adding, or altering sections of the DNA sequence.

Here's how CRISPR/Cas9 is used by scientists to edit DNA:

1. Scientists begin by creating a specific type of RNA, called gRNA.

- **RNA (ribonucleic acid)** is a nucleic acid that "reads" genetic information, and carries that information from DNA to make proteins and regulate genes.
- **gRNA**, which stands for "Guide RNA," locates specific DNA sequences. gRNA is designed to match the specific DNA sequence that needs to be edited.

2. Cas9 binds to the gRNA and uses the RNA sequence to region on the DNA that is complementary. Cas9 then locks onto the double-stranded DNA and unzips it.

3. After the DNA is unzipped, the RNA pairs up with the region of the DNA it has targeted for editing.

4. Next, Cas9 snips the DNA at this spot, creating a break in both strands of the DNA molecule.

- **PAM** stands for "protospacer-adjacent motif," a short sequence of letters in a target DNA strand. There must be a perfect match - *like with puzzle pieces!* - between CRISPR proteins and the PAM of a DNA strand, or the reaction will not occur.

5. The cell will naturally repair the break. In the repair process, the gene that was first targeted for editing may be disabled, or fixed, or in more rare cases, even replaced.

- Because cell repair usually involves fusing the "loose" ends of the cut DNA back together, which is a messy process, mistakes are often made. Mistakes typically result in a gene being disabled, so this is the most common result of gene editing.
- A gene being disabled may not sound beneficial at first, but in the case of genetic diseases, this can actually be quite helpful, or even life-saving!

Summary:
Scientists use CRISPR/Cas9 technology to edit genes, which most often results in a gene being disabled.

Fill in the Blank:

Watch the TED-Ed video, which explains how the revolutionary CRISPR/Cas9 technology works to allow gene editing. During the video, fill in the blanks below with the correct answer from the options provided within the parentheses.

TED-Ed - How CRISPR Allows DNA Editing

1. The _____ *(DNA/RNA)* contained in our genes acts like an instruction manual for our cells.
2. CRISPR is the fastest, easiest, and cheapest of the gene editing tools. CRISPR is a natural process that's long-functioned as a _____ *(regular chemical reaction/bacterial immune system)*.
3. When a virus invades a bacteria, Cas proteins cut out a segment of the viral DNA to stitch into the bacterium's CRISPR region, capturing a chemical snapshot of the infection. A copy of this snapshot, in the form of RNA, then binds itself to a special protein called _____ *(a nucleotide/Cas9)*.
4. The resulting RNA-Cas9 complex then acts like a "scout," patrolling for the virus that has had its snapshot taken. If the virus invades again, the Cas9 recognizes its DNA and _____ *(destroys/mutates)* the virus.
5. In _____ *(1996/2012)* scientists figured out how to use CRISPR to target not only viral DNA, but any kind of DNA.
6. Here's how it works in the lab: Scientists design a _____ *(guide/kinetic)* RNA to match the gene they want to edit, and attach it to Cas9. Instead of snipping an invading virus, this RNA-Cas9 complex instead snips the target DNA. This is the key to CRISPR's power: Just by injecting _____ *(Cas9/viral DNA)* to a short piece of "custom" gRNA, scientists can practically edit any gene in the genome!
7. Once the DNA is cut, the cell will _____ *(rapidly multiply/try to repair it)*.
8. However, if scientists add a separate sequence of template DNA, cellular proteins can perform a different DNA repair process called _____ *(homology/autonomy)* directed repair process. With this type of repair, the template

DNA acts as a blueprint to guide the rebuilding process, repairing a defective gene or even inserting a completely new one.

9. The ability to fix DNA errors means that CRISPR could potentially create new _____ *(phenotypes and genotypes/treatments for diseases)* linked to specific genetic errors.

Draw & Diagram the Key Components of Gene-Editing:

DNA (double strand)	**RNA (single strand)**	**Cas9 (contained the RNA, and snipped the DNA)**

Jennifer Doudna: Co-Discoverer of CRISPR/Cas9 Jennifer Doudna is a biochemist who co-discovered CRISPR/Cas9 gene-editing technology, which allows for calculated changes to DNA. Doudna was awarded the 2020 Nobel Prize in Chemistry for her work, which may one day help contribute to lifesaving treatments for genetic diseases.	

Activity 1: Exploring the CRISPR Machinery (3D Model + Interactive Visualization)

- **Step 1: Introduction to the CRISPR 3D Model**
  With help from your SciTrek Mentor, identify the following parts of the CRISPR Machinery 3D Model:

    - **Cas9 protein**
    - **Guide RNA**
    - **Target DNA**

  Listen closely as your SciTrek Mentor explains the relationships between each component, and how they work together during the CRISPR process. For Step 3, you will use this information to label a diagram here in your workbook.

- **Step 2: Explore the Interactive CRISPR-Cas9 Animation**

  Visit the HHMI BioInteractive CRISPR-Cas9 Interactive at the link below:

  https://www.biointeractive.org/classroom-resources/crispr-cas9-mechanism-applications

  Instructions:

  1. Be prepared to take notes during your time exploring the interactive in the space below:

_____

_____

_____

2. Click **“Start Interactive”** at the top of the page.

3. Click **“How It Works”** to begin exploring the CRISPR process.

4. Use the **“Step”** feature below the animation to go through each stage at your own pace:

    - **Targeting** – How CRISPR finds the correct DNA
    - **Binding** – How Cas9 attaches to the DNA
    - **Cleaving** – How Cas9 cuts the DNA
    - **DNA Repair** – How the cell repairs the break

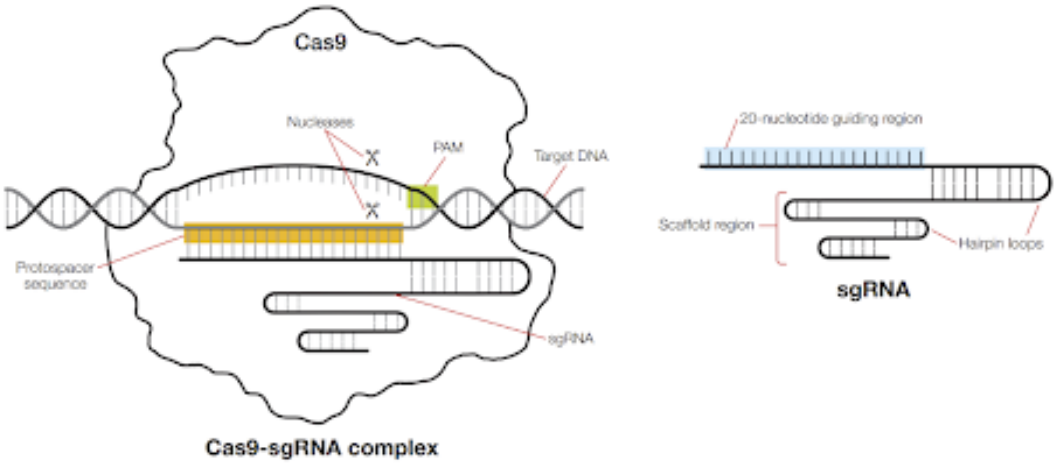
5. Pause after each step and discuss with a partner or small group:

    - What’s happening in this step?
    - What role does Cas9 or the guide RNA play?

6. Scroll down to explore more. Click on the CRISPR components in the model (e.g., Cas9 protein) to learn additional details.

7. Finally, click **“How It Is Used”** to watch a short video on real-world applications of CRISPR in medicine and science.

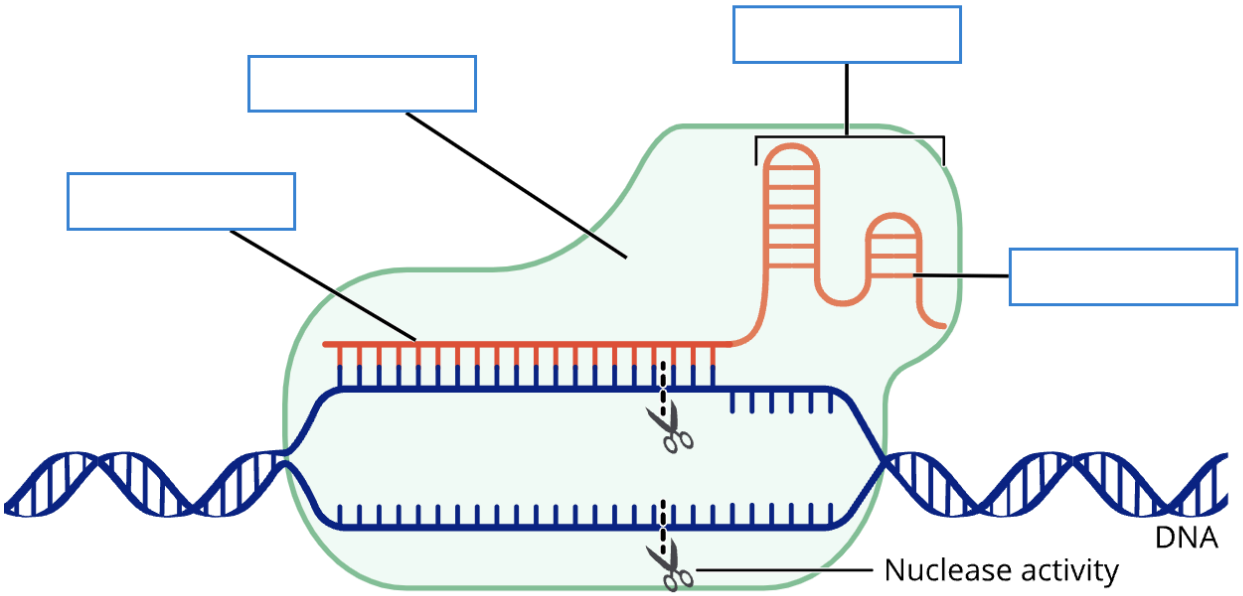
- **Step 3: Make New Connections**
  Revisit the 3D model and workbook diagram. First, verbally identify all of the parts and functions of each piece of the model as shown below.



Next, label the diagram below, according to the provided word bank.

Label the Diagram:

Cas9 Protein	Guiding Region	Scaffold Region	sgRNA



Activity 2: CRISPR Walk-Through

In this activity, you will model the CRISPR-Cas9 system by acting out each component to understand how DNA is targeted, cut, and repaired using a donor template.

Instructions:

Step 1: Setup

- **Get your assigned** role from your SciTrek Mentor, and receive your special props (DNA strands, role cards, scissors, etc).
- Briefly review each role below, and the goal of CRISPR (to locate, cut, and repair a gene) below.

Roles & Functions

Role	Task
DNA Strand(s)	Hold the printed target DNA strand and stretch it across the group
PAM Site	Stand next to the PAM sequence and announce when it's found
sgRNA	Scan the DNA sequence and identify where its matching guide sequence is

Cas9	Follow sgRNA and "cut" the DNA at the target site with scissors
Donor Template	Wait until the DNA is cut, then insert the new genetic material
DNA Repair Crew	Help tape/glue the donor DNA into place and close the strand

Step 2: Pre-Activity Questions

- What do you think makes CRISPR a useful tool for scientists?

_____

_____

- How does CRISPR find exactly where to cut the DNA?

_____

_____

- Why might a cell need help repairing a broken piece of DNA?

_____

_____

Step 3: Simulation

Part 1: Scanning the DNA

- The **DNA students** hold the paper strand stretched across.
- The **sgRNA student** reads along the strand to find the matching guide sequence.
- The **PAM student** announces when a valid PAM site is found adjacent to the target.

Part 2: Cleavage

- Once the match is confirmed, **Cas9** uses scissors to make a single cut at the target site.
- The DNA strand is now "broken."

Part 3: Repair and Edit

- **Donor DNA** steps in, handing the edit strip to the **Repair Crew**.
- The Repair Crew uses tape to attach the new DNA in the place of the old sequence.

Step 4: Questions During the Activity

- What role does the PAM sequence play?

_____

_____

- What happens if there's no PAM sequence next to the target site?

_____

_____

- How does the guide RNA ensure accuracy?

_____

_____

Step 5: Post-Activity Questions

- What might go wrong if the guide RNA is not specific enough?

_____

_____

- In what ways is this activity similar to or different from how CRISPR works in real cells?

_____

_____

- How could this tool be used in medicine, agriculture, or research?

_____

_____

Activity 3: Bacteria Plating Practice

Instructions:

1. **Prepare:** Put on your protective gloves and a face mask before you begin this activity. This is for your safety and the safety of those around you.
2. **Review:** Review the Key Content below on inoculation.

Key Content

During your final Day 1 experiment, you practiced streaking colored "bacterial strains" onto an agar plate. While the colors did not represent real bacteria, each solution symbolized a different simulated strain to help demonstrate the technique of inoculation. The process of introducing microorganisms, such as bacterial strains, into a new medium or environment for growth is called **inoculation**.

Fun Fact: Some people use the term "inoculation" synonymously with "vaccination!" While the term is used in a much broader sense throughout this module, vaccination is a great example of inoculation because it involves introducing inactive viral or bacterial DNA into a new environment (human host) in order to trigger an immune response.

3. **Gather Materials:** Select a petri dish and an inoculating loop from the ethanol solution, shaking the loop a few times until it is mostly dry.

4. **Add Bacterial Solution:** Open the petri dish, and very carefully add 20µL of bacterial solution on top of the gel.

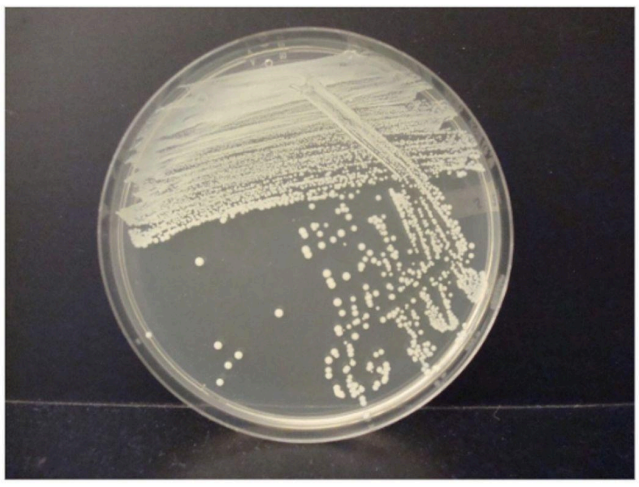
5. **Cover the Gel:** Very carefully place the inoculating loop on the surface of the gel. As a group, verbally divide the plate into four quadrants as you did during your Day 1 quadrant streaking activity. Gently drag the loop across one section in a zigzag motion. Drag the loop from the first streak into a new section (second quadrant), streaking a zigzag again. Repeat for the third and fourth quadrants.

6. **Staging Finished Materials:** Put the inoculating loop back into the ethanol solution, close the petri dish and place it upside down.

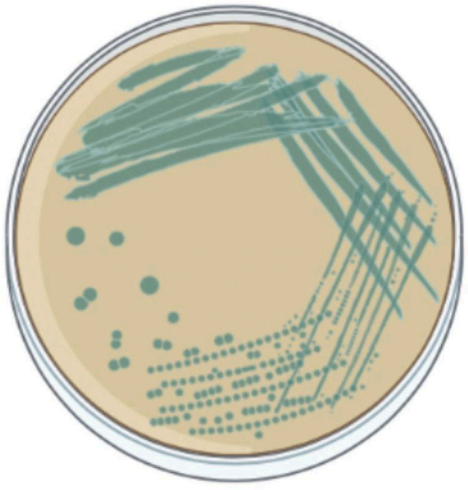
   (Hint: This step helps prevent condensation from changing results.)

7. **Label Your Work:** Label the plate on the bottom edge with your small group's information (table #, period #, name), so that your specific bacterial growth can be tracked and observed over time.

Image of the successful result:



Example of actual bacterial streaking showing isolated colonies (photograph).



Drawing of bacterial colonies on a streaked plate.

Key Questions:

1. Look closely at the streaking pattern in the images above. How does the appearance of the bacteria change from the first streak to the last? Why do you think this change happens?

_____

_____

_____

2. Why do scientists use quadrant streaking when working with bacteria?

   Hint: Think about how it helps in isolating bacteria for research.

_____

_____

3. What is the role of Cas proteins in bacteria or in genetic research?

   Hint: Think about how bacteria naturally use Cas proteins and how scientists use them in gene editing.

_____

_____

4. On Day 1, we plated bacteria using quadrant streaking to isolate colonies. What do you think we are going to do with the individual colonies next?

   Hint: Think about how isolated colonies can be used in experiments.

_____

_____

5. Before we begin Day 2, what do you think we'll need to do to prepare the colonies for testing?

   Hint: Think about what scientists might do after growing bacteria to get results from a gene editing experiment.

_____

_____

Post-Experiments Discussion (Optional)

Use any remaining time at the end of Day 2 for Q&A and discussion with your SciTrek Mentor and small group.

Option 1: Reflect on Activities

Share observations or questions about what your group did today. Think critically about the techniques and concepts used throughout each of the three activities.

Option 2: UCSB College Life Q&A

Ask your SciTrek Mentors any questions you might have about college — especially the day-to-day experiences of being a UCSB student.

Sample Questions You Can Ask:

- *What's your favorite class you've taken at UCSB so far?*
- *What was it like moving into the dorms?*
- *What's something that surprised you about college?*
- *How did you choose your major?*
- *What advice would you give to someone thinking about applying to UCSB?*

Wrap-Up and Reflection

Recap:

- **DNA & Genes:** DNA is a code for making RNA which can then go on to make protein,

and genes are pieces of DNA that code for a single protein.
- **CRISPR/Cas9:** Technology that allows for editing genes within living cells.
- **Small Change, Big Effects:** Even slight edits of genetic code can create big and visible effects.

Reflect:

Think about how even a very small change to a gene - even just one letter base! - can completely change the way that DNA is "read," and the way that its proteins are built. How might these small changes with big effects apply to the modification of genetics in the "real world?"

Hint: Consider genetically-modified crops, and attempts to cure genetic diseases

Preview for Day 3:
Get excited for tomorrow's session where we'll dig deeper into genetic modification and begin the first half of our gene mutation transformation with your UCSB Mentors. See you there!

End of Day 2

Day 3

Beyond the Off-Switch: Exploring Gene Editing with CRISPR

Objective

Today, we will learn how gene editing can change the lacZ gene in E. coli through a hands-on experiment. You will use CRISPR/Cas9 technology to modify E. coli by adding special DNA and guide RNA, observe how these changes affect the gene's function, and gain practical experience using CRISPR. Ready for some cool experiments?

Key Content

In this activity, you will apply CRISPR-Cas9 technology to edit the lacZ gene in *E. coli*. The lacZ gene encodes β-galactosidase, an enzyme that breaks down lactose and also converts X-gal, a colorless molecule, into a blue molecule which you can see on the plate. By interfering with lacZ and observing the color change, you can determine whether or not the gene was edited successfully. Molecular biologists frequently use lacZ as a marker for DNA insertion, taking advantage of the obvious visual contrast between blue and white colonies. In this experiment, you will leverage the blue-white screening technique to visually assess CRISPR-induced gene modifications.

Fun Fact: Universities and research labs use "Blue/White Screening" to engineer bacteria for producing pharmaceuticals, synthesizing industrial enzymes, and studying gene function.

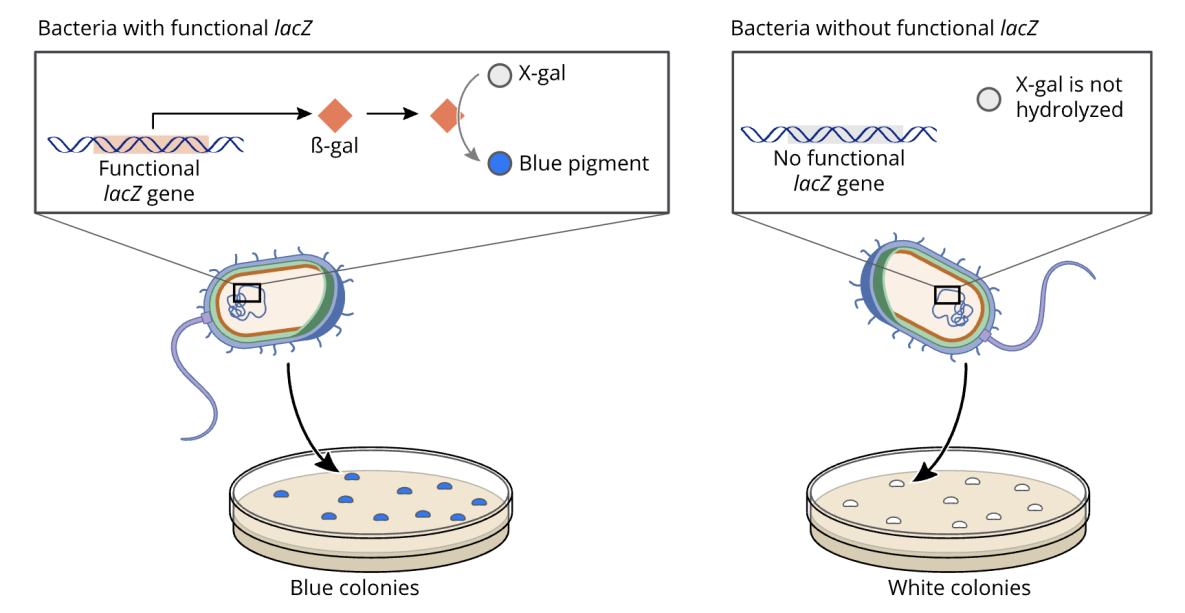
<u>Understanding the Role of Each Reagent</u>

LacZ: This is the gene of interest that CRISPR targets, which codes the enzyme *beta-galactosidase* functioning for the breakdown of lactose into its components (including X-Gal).

X-Gal : X-Gal is a reagent added to all plates. Normally, bacteria with a working **lacZ gene** produce an enzyme called **β-galactosidase,** which breaks down X-Gal and turns the colonies **blue**. However, if CRISPR knocks out the lacZ gene—meaning it makes a change in the DNA that stops the gene from working—the bacteria can't produce

β-galactosidase. Without the enzyme, X-Gal stays unchanged, and the colonies remain **white**.

Think of X-Gal as invisible ink—it needs β-galactosidase (the "magic pen") to reveal a blue message. If CRISPR removes the magic pen by disrupting the lacZ gene, the ink stays invisible, and the colonies stay white!



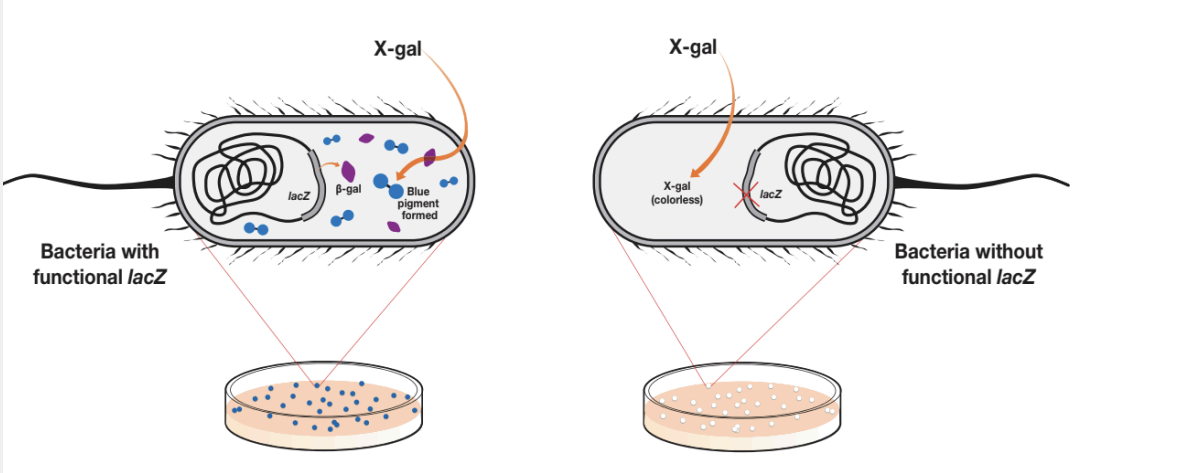
Kanamycin: An **antibiotic** is a substance that **kills or stops the growth of bacteria** by targeting essential processes like protein synthesis or cell wall formation. In this experiment, **kanamycin** is the antibiotic used. It **blocks protein synthesis in bacteria that do not have resistance to it**, preventing them from growing. To ensure that only **HB101-pBRKan E. coli** bacteria grow on the plates—and not mold or other unwanted bacteria—kanamycin is added to all plates. These E. coli have a **plasmid (pBRKan) that provides resistance to kanamycin**, allowing them to survive while other bacteria are eliminated.

IPTG: induces the expression of the CRISPR/ CAS9 proteins that make the cuts in the LacZ gene downstream of the PAM site, allowing for active CRISPR function

Arabinose: A type of sugar that acts as a switch to turn on the production of special repair proteins. These proteins help the bacteria fix the DNA cuts made by CRISPR/Cas9 proteins through a process called Homologous DNA Repair (HDR).

Spectinomycin: An antibiotic that kills most bacteria, but the E. coli used in this experiment are resistant, meaning they can survive while unwanted bacteria are eliminated.

Transformation Solution ($CaCl_2$ - Calcium Chloride): A chemical solution that helps bacteria take in foreign DNA. It creates tiny holes in the E. coli cell membrane, allowing the plzDonorGuide plasmid (which carries the CRISPR instructions) to enter the bacteria and begin gene editing.



Understanding Key Terms:

Plasmid: A small, circular piece of DNA found in bacteria. It's separate from the bacteria's main DNA and can carry extra genes, like ones for antibiotic resistance. Scientists often use plasmids in genetic engineering to insert new genes into bacteria.

Positive Control: a sample treated with a known factor that should produce the ideal result.
Negative Control: a sample treated/untreated with a known factor that should not produce the ideal result.

Activity 1: Gene Editing with CRISPR-Cas9 technology

The goal of today's experiment will be to make correct and thoughtful predictions on which mix(es) of substances will result in the successful function of CRISPR/Cas9. In this case, successful function will be a gene "knockout," which means that CRISPR will cut and deactivate the gene that causes the bacteria to turn blue in color. The colonies with active CRISPR function will be white, indicating that the gene has been successfully turned off!

Instructions:

1. **Prepare:** Put on your protective gloves and a face mask before you begin this activity. This is for your safety and the safety of those around you.
2. **Organize:** Label four 2.0 ml microcentrifuge tubes A–D and place on ice.
3. **Dispense TS:** Using a P1000 micropipette, add 250µl ice cold transformation solution (TS) to each test tube. Place the test tubes back on ice.
4. **Sterilize:** Sterilize the inoculation loop by swirling the instrument in the solution of 70% ethanol, and with the help of your SciTrek Lead, quickly passing it through a flame.
5. **Inoculate:** Add the following substances to each test tube, making sure that each group member has at least one chance to dispense material:
    - (TUBE A)

      HDR negative control group (repair, no guide): Using the sterilized loop, collect 5 colonies from the KIX plate and swirl into the repair negative control microtube (A). Then add 10µL of the **plzDonor plasmid**. Flick 3-5 times to mix. Resterilize.

    - (TUBE B)

      CRISPR/CAS9 cutting control group (with guide): Using the sterilized loop, collect 5 colonies from the KIX plate and swirl into the CRISPR cutting control group microtube (B). Then add 10µL of the **plzDonorGuide plasmid**. Flick 3-5 times to mix. Resterilize.

    - (TUBE C)

      CRISPR/Cas9 LacZ Knockout negative control group (no guide): Using the sterilized loop, collect 5 colonies from the KIX+A re-streak plate and swirl into (-) control tube (C). Then add 10µL of the **plzDonor plasmid**. Flick 3-5 to mix. Dispose of inoculation loop.

- (TUBE D)

  CRISPR/Cas9 LacZ Knockout positive control group (with guide): Using the sterilized loop, collect 5 colonies from the KIX+A re-streak plate and swirl into (+) control tube (D). Then add 10 µL of the **plzDonorGuide plasmid**. Flick 3-5 to mix. Dispose of inoculation loop.

6. **Wait:** Start a timer, and let the test tubes sit on ice for 10-20 min without disturbing the solution.
7. **Heat Shock:** With help from your SciTrek Lead, heat shock the test tubes at 60°C for 50 seconds, making sure the bottoms of the tube are fully submerged.
8. **Cold Plunge:** Immediately put the test tubes back on ice for 2 minutes.
9. **Add Luria Broth (LB):** With a new pipette tip, add 250µL of LB broth to each tube. Flick the tube three times to mix.
10. **Wait:** Leave the test tubes at room temperature for 20 minutes.
11. **Label:** Label four plates A-D with your initials and date along the corner of the base.
12. **Remix & Dispense:** Flick tube A to resuspend the bacteria. Then, pipette 100µl of Tube A onto Plate A.
13. **Spread Solution:** Making sure to not pierce the agar surface, gently spread the solution using a new inoculation loop. Rotate the plate to evenly spread it.

    Using a new pipette tip AND a new inoculation loop for each sample, repeat this step for plates B-D.

14. **Cover & Store:** Cover, stack, and tape the plates. Incubate them upside down at 37°C.
15. **Hypothesize:** Using the REAGENTS & GUIDES INFO TABLE below, discuss your expected results with your group and make a hypothesis for the results that you will observe on Day 4.

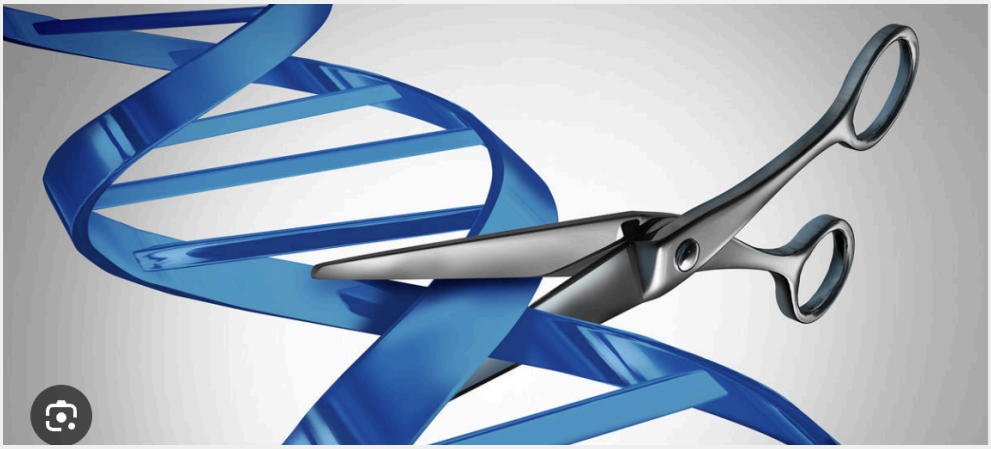
REAGENTS & GUIDES DATA TABLE

Plates	**Reagents**	**PlasmidDonorGuide/ PlasmidDonor**
A	IPTG, X-gal, spectinomycin	PlasmidDonor
B	IPTG, X-gal, spectinomycin	PlasmidDonorGuide
C	IPTG, X-gal, arabinose, spectinomycin	PlasmidDonor
D	IPTG, X-gal, arabinose, spectinomycin	PlasmidDonorGuide

Key Content

What is the difference between a PlasmidDonor vs a PlasmidDonor Guide? How do they work?

Imagine CRISPR/CAS 9 as a pair of DNA scissors!



With a guide: The scissors know *exactly* where to go, and what cuts - *resulting in genetic changes* - to make. The guide RNA tells the Cas9 where it should be cut. This shows activation of CRISPR/Cas9 machinery. Then the DNA repair system is activated to fix this cut!

Without a guide: The scissors have *no clue* where to go, or what cuts should or shouldn't be made. They may accidentally cut something important and damage a gene, or most likely, just not make a cut at all! This renders CRISPR machinery ineffective!

Key Questions:

1. Why Choose CRISPR over other gene editing methods?

_____

_____

_____

2. What is protein expression? (Central Dogma Recap)

_____

_____

_____

_____

3. Provide a definition for both bacterial growth and protein expression. Can one happen without the other? Explain.

Term	What it means
Growth	
Protein Expression	

_____

_____

_____

_____

4. In your own words, provide a definition for each of the following and its special function in this experiment.

Component	What it is & How it's Used
IPTG	
X-gal	
Kanamycin	
Spectinomycin	
Plasmid Donor	
Plasmid Donor Guide	
Arabinose	

5. Experimental Design Chart (for Hypothesis & Setup): Check off which components are present on each plate, then predict what you expect to observe on that plate.

Plate	IPTG, X-gal, kanamycin, spectinomycin	**X-gal**	**Arabinose**	**Plasmid Donor**	**Plasmid Donor Guide**	**What will happen?**
A						[Prediction]
B						[Prediction]
C						[Prediction]
D						[Prediction]

6. For each sample (A–D), state whether you expect to observe growth, no growth, or evidence of CRISPR gene editing. Explain.

Growth (Blue Colonies):

_____

_____

No Growth:

_____

_____

Successful CRISPR Editing (White Colonies):

_____

_____

7. Create a hypothesis for which plate(s) will show successful CRISPR function (color knockout), and why.

_____

_____

_____

_____

8. What is the purpose of including both a positive and negative control in this experiment?

_____

_____

_____

_____

9. Why is it important to use sterile technique when working with bacteria?

_____

_____

_____

_____

Wrap-Up and Reflection

Recap:

- **Gene Knockout:** A genetic engineering technique that inactivates, or deletes, a specific gene in an organism.
- **gRNA:** The guide for the "DNA scissors" that is CRISPR/Cas9. Without gRNA, CRISPR will either make incorrect changes or fail to make any changes whatsoever.
- **Reagents & gRNA:** In this experiment, two factors were considered as you formed your hypothesis - 1) Each sample's unique mix of reagents and 2) The presence or absence of gRNA.

Reflect:
Genetic modification is tricky, because CRISPR/Cas9 must locate and match-up with its specific sequence in order for the correct changes to be made. Multiple factors affect whether or not CRISPR will actively function. Why might this be strongly taken into consideration when creating laws around the use of CRISPR in more complicated organisms, particularly mammals?

Preview for Day 4:
Get excited for tomorrow's session where we'll determine whether or not today's hypothesis was correct, and analyze the effects of our reagent mixes and the presence or absence of gRNA on our attempts at gene knockout. See you there!

End of Day 3

Day 4

Masterwork: Analyzing Gene Editing Outcomes

Welcome back, scientists! Today, you're going to discover more about genetic modification with a brand new goal in mind: analyzing the results of yesterday's gene knockout activity. It's time to draw important conclusions about the success of your experimental choices, and compare with those of your classmates!

Objective

You will continue your journey in genetic modification by analyzing your group's results. You will draw important conclusions about the success of your experimental choices, and compare with those of your classmates. On Day 5, you'll turn this information into a research-style slideshow presentation to share with the class. Ready to begin?

---

1. Recap & Review

Think back to the past days' experiments:

- **Bacteria Plating:** On Day 1, you learned some helpful techniques for micropipetting & bacteria plating that you later used Days 2 &3.
    - Were there any "best practices" that you had forgotten by the later days?
    - Which techniques were hardest to master, and how might this have affected your results?
- **Genetic Transformation:** Although everyone used the same reagents and plasmids, different groups may have observed different results. Let's revisit why that could be.
    - How does the DNA introduced to the bacteria lead to protein expression? How does the DNA introduced into the bacteria lead to the production of β-galactosidase, which causes the blue color on X-gal plates?

- How does the central dogma (DNA → RNA → Protein) explain why some bacterial colonies turned blue while others stayed white?

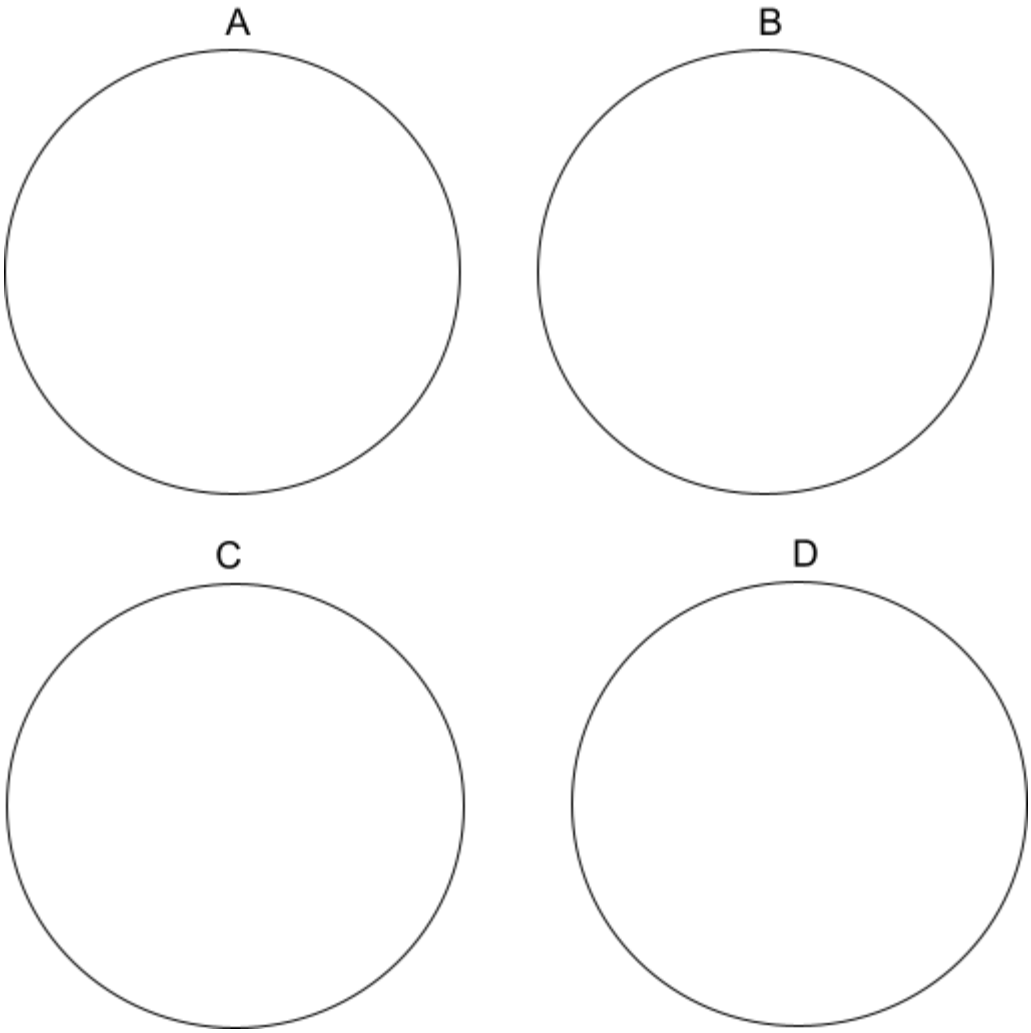
- What happens if the RNA transcribed from the inserted DNA contains a stop codon early in the sequence?

Activity 1: Petri Dish Predictions

List the reagents added to each Petri dish (A–D), then draw what you expect to see after incubation. Be sure to use blue if you expect the lacZ gene to be expressed — this indicates a functional outcome.

Hint: Use your chart of plate contents to guide your predictions.

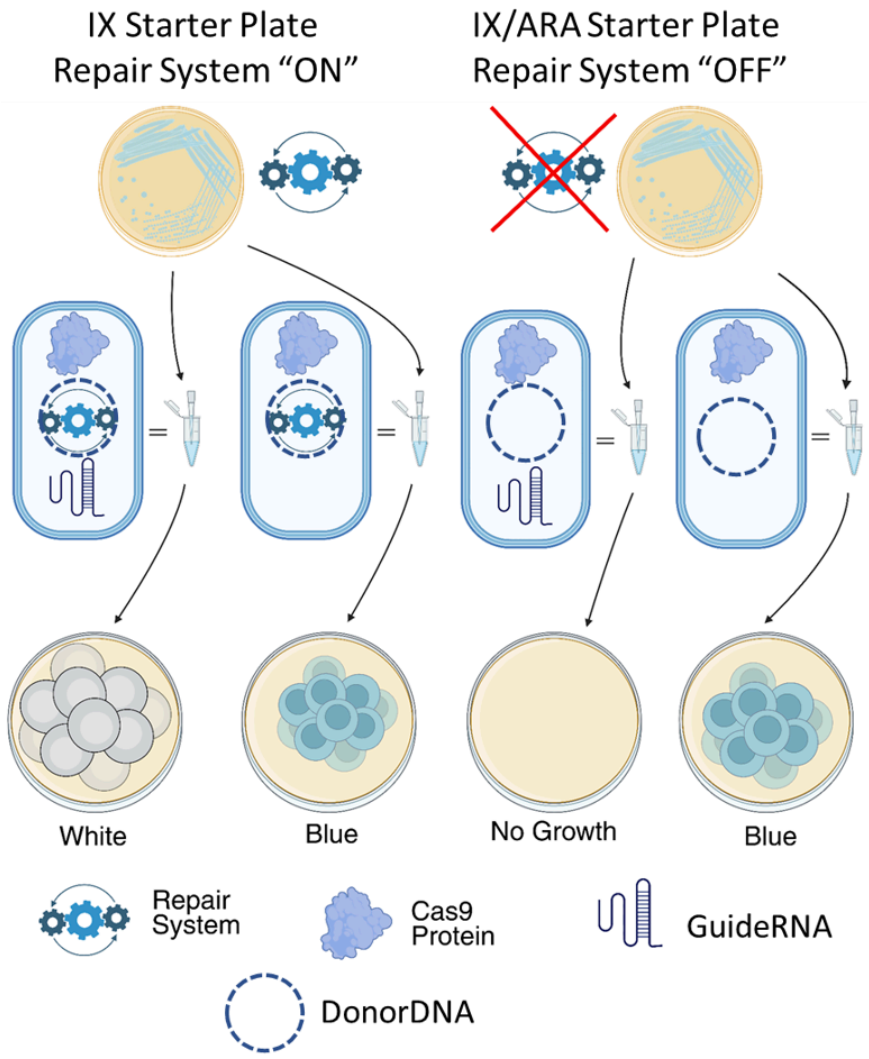
Petri Dish	***Reagents Added***
Petri Dish A	
Petri Dish B	
Petri Dish C	
Petri Dish D	



Your Mission:

In order to analyze your results and draw meaningful conclusions from your culture, you will first need to record your data in an organized manner.

Here's what we expect…



KIX: Abbreviation for the plate containing **kanamycin, IPTG, and X-Gal.**
KIX/ARA: Abbreviation for the plate containing **kanamycin, IPTG, X-Gal, and arabinose.**

Key Questions:

1. Work together as a group to **fill out the data table below:**

REAGENTS & GUIDES DATA TABLE

Plates	Reagents	PlasmidDonorGuide/ PlasmidDonor	Growth? (yes/no)	# of Colonies	Color? (white/blue)
A	IPTG, X-gal, spectinomycin	PlasmidDonor			
B	IPTG, X-gal, spectinomycin	PlasmidDonorGuide			
C	IPTG, X-gal, spectinomycin, arabinose	PlasmidDonor			
D	IPTG, X-gal, spectinomycin, arabinose	PlasmidDonorGuide			

2. **Were the culture's color and growth what you had predicted?** Explain.

_____

_____

_____

3. Were there any steps during your experimental process that you suspect could have **negatively affected your results, or caused some degree of error?** If yes, provide specific examples:

_____

_____

_____

4. **Would you do anything differently** if offered the chance to conduct another trial? Explain.

_____

_____

_____

5. Take a photo of your final culture, and be prepared to use it in tomorrow's slideshow.

Activity 2: Analysis of Class Results

Key Questions:

1. Record the **IPTG, X-gal, spectinomycin, arabinose (Plate B)** culture data from all groups into the class data table below. This will allow you to compare results and knockout effectiveness.
    a. Circle or highlight the row in the data table made by your small group.
    b. ***Note:*** *Some classes may not have six groups, depending on your class size. This is okay! Just ignore the extra rows.*

SAMPLE CLASS DATA TABLE

Group Name/ Number	Growth? Y/N	# of Colonies	Color
1			
2			
3			
4			
5			
6			

2. Which KIX/Ara (IPTG, X-gal, spectinomycin, arabinose) culture had the **most effective gene modification?** Explain.

(Hint: You should reference both color and number of colonies!)

_____

_____

_____

3. Which cultures **did not experience effective gene modification?** Describe the results for these plates.

   (Hint: You should reference both color and number of colonies!)

_____

_____

_____

4. **Explain the reason that the three other cultures (Plates A, B, C) did not experience success** in terms of their genetic modifications.

_____

_____

_____

_____

Activity 3: Discussion on Real-World Application & Ethics

Your Mission:
Learn about the ethical concerns behind genetic modification, and think critically about how we ought to balance inventiveness vs. safety vs. impacts on society.

Key Content

While CRISPR/Cas9 inspires hope and may bring healing for those born with genetic diseases, this new technology also raises a lot of ethical questions and concerns. While gene modification may soon become more available for human use, we will first need to

think deeply on how it should be used with regard to what is morally right for society.

For example, CRISPR/Cas9 is already being tested as a **gene therapy tool** for genetic diseases. Diseases such as sickle cell anemia, cystic fibrosis, and Huntington's disease - all of which have severe effects on a person's life - are a major focus when it comes to this technology.

Despite its many potential benefits as a gene therapy tool, however, CRISPR-Cas9 technology may also make significant errors that could be quite dangerous. In some cases, CRISPR/Cas9 may mistakenly target and cut at sites in the genome that are incorrect.

Remember, DNA is like an instruction manual for cells, and even the smallest change can completely alter the way these instructions are read by our bodies. So while the use of CRISPR/Cas9 as a gene therapy tool has huge potential for healing, it may also have serious risks.

Summary:
Scientists may soon begin to use CRISPR/Cas9 to edit human genes, which has potential for hugely positive effects, but also risk for serious negative effects. This raises many ethical questions about how CRISPR/Cas9 should be permitted to be used in the medical community.

CRISPR/Cas9 could also be used for genetic modification based solely on preference. You can think of this potential use for genetic modification as **designer genes**. At first, this idea may sound exciting! Have you ever wished that you could change your eye or hair color, or give yourself freckles or dimples? Perhaps you have dreamed of greater muscle mass, or clearer skin. Designer genes could make all this and more possible!

However, the possibility of designer genes also comes with a serious set of ethical questions that affect individuals, future generations, and society as a whole. For example, designer genes are often considered with the potential for parents to "design" a future baby's traits through genetic modification. Parents might make selections for all sorts of preferences such as eye color, hair color, athleticism, height, skin pigmentation, or even gender. Many argue that this would be problematic for two reasons:

1) **Gene Selection & "Enhanced" Status:** Because designer genes would be simply based on preference, this technology would likely be quite expensive. Trendiness and privilege may become associated with certain traits that only the

wealthy can ensure for their children, while children of families with a lower socioeconomic status may be marginalized for lack of "enhanced," traits.

2) **Gene Modification & Natural Integrity:** Parents should not have this level of authority over these irreversible characteristics of their babies. This interferes with the natural integrity of the baby that they are planning to have, and may not be desired by the person living with those modifications in the future.

Summary:
CRISPR/Cas9 could be used to modify certain traits based solely upon preference, otherwise known as "designer genes." The capability of designer genes raise many ethical questions about how hand-picked traits could affect both societal structures and personal rights.

Key Questions:

1. If you were designing a CRISPR-based therapy for a genetic disease, **why would "accidental cuts" be a major ethical concern**? What steps could you take to minimize these risks?

_____

_____

_____

2. If a moderate amount of **risk is unavoidable, should the technology still be available to patients?** Why, or why not?

_____

_____

_____

3. **How could genetic enhancement improve people's lives?**

_____

_____

_____

4. Overall, would you prefer **a society where gene modification based solely on physical preferences ("designer genes") was a widely available option?** Why or why not?

_____

_____

_____

5. Governmental agencies have very strict guidelines on CRISPR research and experimentation, especially with mammals. Imagine that you are advising the US president on CRISPR research - **How would you recommend balancing inventiveness and safety?**

_____

_____

_____

Group Discussion & Wrap-Up

- **Bonus Question:** If the most effective plate were to still show a significant number of blue colonies (unmodified physical traits), what would be two possible explanations for this incomplete modification?

_____

_____

_____

Conclusion and Reflection

- **Wrap-Up:** Today, analyzed your results from Days 2 & 3, prepared your findings for presentation tomorrow, and explored important ethical questions surrounding the future use of CRISPR/Cas9 technology. Great job on your hard work!
- **Final Thought:** What was the biggest challenge you faced, and what did you learn from it?

Write your reflection here:

End of Mission! Ready for Day 5? On this final program day, you will demonstrate what you learned earlier this week.

End of Day 4

Day 5

Poster Presentation: Showcasing Your Scientific Journey!

Hey, scientists!

Today, you get to show off all the hard work you did during your "Beyond the Off-Switch: CRISPR Knockout" experiment by creating a slideshow in the style of a research poster. The slideshow will help you share your findings in an engaging and interesting way, just like real scientists do at conferences. Let's get started on making your scientific findings stand out!

Introduction: Why Make a Slideshow in the Style of a Research Poster?

- **Did you know?** Scientists use posters to share their experiments and discoveries with others. Your mission today is to create a slideshow in the style of a research poster that tells the story of how you experimented with genetic modification and knockout strategies.
- **What goes on the poster?** You'll need a catchy title, a hypothesis, the procedure you followed, a picture of your final culture, your results, and a conclusion.

Slideshow Creation

You'll plan your slides here in your workbook, then transfer the information to your own slideshow template. Take your time and have fun! This is your opportunity to show off your creativity and individuality as a scientist.

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Hint: The QR code below will show you a template that you can use as inspiration for the design of your own presentation!

Step 1: Title and Hypothesis

- **Title Time:** Come up with a clear and interesting title for your project. Make sure it reflects what your experiment was all about.
    - *Example:* "K.O. - Gene Modification to Knock Out the Blues"
- **Hypothesis:** Write down what you were testing for in your experiment, and what your group believed the result would be.
    - *Example:* "We tested for the effectiveness of the reagents and gRNA on modifying the genes of a bacteria that create proteins for coloration. We believed that the result would be mostly white colonies, with just a few blue colonies mixed-in."

Question: What's your title?

_____

_____

Question: What was your hypothesis?

_____

_____

Step 2: Description of Procedure

- Write a short explanation of the experiment's procedure, with emphasis on the purpose of each step in the genetic modification process.

_____

_____

_____

_____

Step 3: Picture of the Final Culture

- **Get Creative:** Take a photo of your final culture if you haven't already, or draw a picture of it and take a photo of your drawing.
- Upload the photo to your device, and add it to your slide.

- On the following slide, write a short explanation of the photo including all information from your first data table on Day 4:
    - Plasmid Description & Plate (A, B, C, D)
    - Growth? Y/N
    - # of Colonies
    - Color

Step4: Results

- **What did you find?** Summarize the results of your experiment, including your group's expected results and your actual results, the effectiveness of your knockout strategy compared to other groups, and why your group's results were or were not as expected.
- On the final two slides, explain what your group sees as the biggest ethical benefit of the CRISPR/Cas9 technology, and the biggest ethical drawback.

Step 5: Conclusion

- **What does it all mean?** Write a conclusion that explains your results. Did your results support your hypothesis? Why or why not?
    - Explain in terms of genetic modifications & color knockout.
- **Think about it:** Discuss any challenges you faced, potential sources of error, and ideas for improving your design next time.

Question: What is your conclusion?

Step 6: Assemble the Slideshow

- **Put it all together:** Use the structural guidelines above to create neat slides for all of the elements of the slideshow. Organize your information logically—make sure everything is clear and easy to read.
- **Get Creative:** While your slideshow should be neat and easy to follow, feel free to add some flair with colors and designs!

Slideshow Presentation

Presentation & Peer Feedback

- **Showtime:** Display your finished slideshow for the rest of the class, one small group at a time.
- **Feedback:** Give and receive positive feedback. What did you like about other slideshows? What could be improved for next time?

Conclusion and Reflection

- **Wrap-Up:** Reflect on the slideshow-making process. Why is it important to communicate your scientific findings effectively?
- **Looking Ahead:** Think about how you can use these skills in future projects and presentations. Great job today!

Final Reflection: What did you learn from making your slideshow?

End of Day 5!

You did amazing—keep up the great work, and stay curious!

SciTrek is an educational outreach program that is dedicated to allowing 2nd - 12th grade students to experience scientific practices firsthand. SciTrek partners with local teachers to present student-centered inquiry-based modules that not only emphasize the process of science but also specific grade level NGSS performance expectations. Each module allows students to design, carry out, and present their experiments and findings.

If you would like to donate to the program or find out how you can get your company's logo on our notebooks please contact scitrekadmin@chem.ucsb.

SciTrek is brought to you by generous support from the following organizations:

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