The Power of CRISPR
Workshop Goals

● Gain familiarity with the *Power of CRISPR* unit
● Understand how students construct their understanding of the mechanism of the CRISPR-Cas9 system and how it can be incorporated into high school life science content.
● Gain interest and excitement in incorporating cutting edge research and current issues into your classrooms.
Agenda

- Introduction
- Overview
- Activity Highlights
  - Hands-on model
  - Laboratory Experiment
  - Articles and Ethics
- Closing
Unit Overview

● Students learn about how CRISPR works in the context of using it to treat sickle cell disease, a genetic blood disorder.

● Main Learning Goals:
  ○ Genes → Proteins → Traits
  ○ CRISPR is a method of gene editing that allows scientists to insert, delete, or change sections of DNA in order to change an organism's traits.

● NGSS PEs HS-LS1-1 (DNA → Proteins) and HS-ETS1-1 (addressing challenges)
# 6 Lesson Unit

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What comes with the unit?

Teacher’s Guide

Student Facing Materials

Videos and Slides

Hands-On and Lab Materials
Intro to CRISPR Video

- Will be available soon at https://sepuplhs.org
Modeling Sickle Cell

Part 1
Sickle cell disease is caused by a mutation in a gene called the hemoglobin beta gene, one of the genes that codes for hemoglobin, a protein that carries oxygen in the blood.

Complete the following activity to better understand how the sickle cell mutation affects the hemoglobin process. In this model, different-colored beads represent different amino acids. A string of amino acids is a protein. The spots on paper with the code represent the genes.

Directions
1. tRNA and ribosomes are parts of the cell that help build proteins, using the DNA as a code. Decide which partner will be the tRNA and which partner will be the ribosome.
   - The tRNA will translate and read the code.
   - The ribosome will build the protein.
2. tRNA: Translate the hemoglobin beta gene using the following code (1 = yellow, 2 = blue, 3 = red, 4 = black). Write the colors on the gene strip according to the code.
3. tRNA: Read the order of the amino acids and give the ribosome each amino acid in that order.
4. Ribosome: Create the hemoglobin beta protein by threading the amino acids (beads) onto the pipe cleaner in a straight chain as the tRNA hands them to you. Bend the ends of the pipe cleaner to keep the beads in place.
5. tRNA and ribosome: Repeat Steps 2–4 with the mutated hemoglobin beta gene to build the sickle hemoglobin beta protein.

Questions
What was the role of the gene in building the protein?

Compare the two proteins. How are they different? What led to the difference in the proteins?

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Modeling Sickle Cell

Use the code to read the DNA sequences:

- GAG = yellow
- GTG = blue
- TCC = red
- GTA = black
Example completed models
Students gather additional evidence about the causes of sickle cell disease by reading an article.

Sickle Cell Disease

Introduction

Sickle cell disease is an inherited blood disorder that is caused by stiff and irregularly shaped red blood cells. Their shape looks like a crescent moon, or sickle, which is where the disease gets its name. Typically, red blood cells are smooth and round, allowing them to easily glide through blood vessels throughout the body. The reshaped red blood cells in people with sickle cell disease do not glide through the blood vessels like the smooth, round ones. They tend to pile up, blocking proper blood flow and preventing oxygen from getting to vital organs and tissues. This leads to fatigue, episodes of pain, swelling of the hands and feet, and frequent infections. Complications such as stroke and organ damage can even lead to death.
Read, Think, and Take Note

As you read, from time to time, use a sticky note to do one of the following:

• Explain a thought or reaction to something you read.

• Note something in the reading that is confusing or unfamiliar.

• List a word that you do not know.

• Describe a connection to something you learned or read previously.

• Make a statement about the reading.

• Pose a question about the reading.

• Draw a diagram or picture of an idea or connection.

After writing each sticky note, place it next to the word, phrase, sentence, or paragraph in the reading that prompted your note.
Introduction to the CRISPR Lab

- Video available with purchase of the kit, at https://www.lab-aids.com
CRISPR Lab Overview

Part 1
Add bacteria to liquid LB

Wait 24 hours

Part 2
Activate CRISPR genes

Wait 24 hours

Part 3
Move bacteria to agar dishes

Wait 72 hours*

Observation
Observe bacteria

Everything at room temperature!
No special equipment? No problem!
PLAN AHEAD!
Follow the protocol!

* Growth of bacterial cells on agar generally takes 72 hours at room temperature, but can vary from room to room.
Conducting the CRISPR Lab

- Video available with purchase of the kit, at https://www.lab-aids.com
Curing Sickle Cell Using CRISPR

- Will be available soon at https://sepuplhs.org
# How Does CRISPR Work?

<table>
<thead>
<tr>
<th>Component</th>
<th>What role does it play in the CRISPR system?</th>
<th>Is this component the same or different in the bacteria lab and in humans with sickle cell disease? Explain.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle Cell Mutation</td>
<td></td>
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<tr>
<td>Hemoglobin Gene</td>
<td></td>
<td></td>
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<tr>
<td>Cas9</td>
<td></td>
<td></td>
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<tr>
<td>Guide Sequence</td>
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<tr>
<td>Repair Protein</td>
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<tr>
<td>Donor DNA</td>
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</table>
Gene Editing in Bacteria and Humans

What is the target DNA sequence for CRISPR editing in your lab experiment? What is the target DNA sequence for CRISPR editing in sickle cell disease?
### Comparing CRISPR Components

<table>
<thead>
<tr>
<th></th>
<th>Target Gene</th>
<th>Guide Sequence</th>
<th>Donor DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Our lab experiment</strong></td>
<td><img src="image1" alt="RFP GENE DNA diagram" /></td>
<td><img src="image2" alt="GUIDE SEQUENCE" /></td>
<td><strong>GFP DONOR DNA</strong></td>
</tr>
<tr>
<td><strong>Sickle cell disease</strong></td>
<td><img src="image3" alt="HEMOglobin GENE SICKLE CELL MUTATION" /></td>
<td><img src="image4" alt="GUIDE SEQUENCE" /></td>
<td><strong>DONOR DNA</strong></td>
</tr>
</tbody>
</table>
Lab Results

Non-targeting (control)        Targeting (experimental)
Lab Prediction Questions

The bacteria need to multiply before you can see the trait for color. Answer the questions below to predict what the bacteria’s traits will be.

What color will the bacteria from the “Control” tube be after they multiply on the agar petri dish? Explain your answer.

What color will the bacteria from the “Experimental” tube be after they multiply on the agar petri dish? Explain your answer.
UV Light-Viewing Boxes

Insert the petri dish.

Shine the UV flashlight onto the dish and view the dish through the orange film.
Ideal Lab Results

Control: Unedited cells  Experimental: Edited cells
Other Lab Results

A pink color in the middle of a dense layer of green cells can be caused by transferring too much bacteria during the previous steps or by bacteria growing a little more than expected.

In areas of dense cell growth, the red fluorescence from a small number of unedited cells can overpower the comparatively weaker green fluorescence from the edited cells.

Control: Unedited cells  Experimental: (Mostly) edited cells
Completed Model

START

The RFP gene gives the cell instructions to make the red fluorescent protein which gives the bacteria the red glowing trait.

RFP gene
DNA
red fluorescent protein
bacteria with red glowing trait

REPAIR PROTEINS

GFP donor DNA

Once the gene is cut, repair proteins use the donor DNA to repair the break. The donor DNA is the GFP gene.

Cas9

Cas9 matches the RFP gene, allowing Cas9 to find the RFP gene.

guide sequence
RFP gene

Cas9 then cuts the gene.

END

GFP gene

The GFP gene provides the cell with instructions to make the green fluorescent protein which gives the bacteria the green glowing trait.

GFP gene

Now the RFP gene has been replaced by the GFP gene in the bacterial DNA.

Green fluorescent protein
bacteria with red glowing trait

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Articles: Ethics of CRISPR

- Treating Sickle Cell Disease
- Preventing Cystic Fibrosis
- Fighting Malaria
- Disease-Resistant Rice

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Ethics of CRISPR

Under What Circumstances Should CRISPR Be Used?

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Treating Sickle Cell Disease

Should CRISPR be used to treat humans with diseases?

- What is the biggest risk?
- What is the biggest benefit?
- Do the risks outweigh the benefits, or do the benefits outweigh the risks?

Image credits:
Patient with mask: tiverylucky/Shutterstock.com; Patient in bed: Thaiview/Shutterstock.com
Should CRISPR be used to edit genes in embryos, sperm, or eggs?

- What is the biggest risk?
- What is the biggest benefit?
- Do the risks outweigh the benefits, or do the benefits outweigh the risks?

Image credits:
Human egg cells: 895Studio/Shutterstock.com; Babies: sirtravelalot/Shutterstock.com
Fighting Malaria

Should CRISPR be used to edit genes in insects to prevent the spread of disease?

- What is the biggest risk?
- What is the biggest benefit?
- Do the risks outweigh the benefits, or do the benefits outweigh the risks?
Disease-Resistant Rice

- What is the biggest risk?
- What is the biggest benefit?
- Do the risks outweigh the benefits, or do the benefits outweigh the risks?
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