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 $\rightarrow$ Proteins $\rightarrow$ 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 $\rightarrow$ Proteins) and HS-ETS1-1 (addressing challenges)
# 6 Lesson Unit

<table>
<thead>
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<th>Lesson 1</th>
<th>Lesson 2</th>
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<th>Lesson 4</th>
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<tbody>
<tr>
<td>Intro to CRISPR and Sickle Cell</td>
<td>Lab Part 1 &amp; CRISPR Overview</td>
<td>Lab Part 2 &amp; CRISPR Details</td>
<td>Lab Part 3 &amp; Acting Out CRISPR</td>
<td>Lab Results &amp; Modeling Understanding</td>
<td>Ethics of CRISPR</td>
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What comes with the unit?

Teacher's Guide

Student Facing Materials

Videos and Slides

Hands-On and Lab Materials

Unit Overview

What comes with the unit?

Teacher’s Guide

Student Facing Materials

Videos and Slides

Hands-On and Lab Materials

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Intro to CRISPR Video

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

### Hemoglobin Beta Gene

<table>
<thead>
<tr>
<th>gene</th>
<th>3 3 3 1 1 3 3 1 4</th>
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<tbody>
<tr>
<td>amino acid (beads)</td>
<td>3 3 1 1 3 3 3 1 3</td>
</tr>
</tbody>
</table>

### Mutated Hemoglobin Beta Gene

<table>
<thead>
<tr>
<th>gene</th>
<th>3 3 3 1 1 3 3 2 4</th>
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<tbody>
<tr>
<td>amino acid (beads)</td>
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</tbody>
</table>

**Part 1**

Sickle cell disease is caused by a mutation to 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 protein. In this model, different colored beads represent different amino acids. A string of amino acids is a protein. The steps of paper with the beads 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.
2. The tRNA will translate and read the code.
3. The ribosome will build the protein.

2. **tRNA**: Translates 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 ribosomes**: Repeat Steps 2–4 with the mutated hemoglobin beta gene to build the sickle hemoglobin beta protein.

**Questions**

1. What was the role of the gene in building the protein?
2. Compare the two proteins. How are they different? What led to the difference in the protein?
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.
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.
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 takes 72 hours at room temperature. This process can be sped up at higher temperatures.
Introduction to the CRISPR Lab

- Video available with purchase of the kit, at https://www.lab-aids.com
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>HEMOGLOBIN GENE</td>
<td></td>
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<tr>
<td>SICKLE CELL MUTATION</td>
<td></td>
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<tr>
<td>CAS9</td>
<td></td>
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<tr>
<td>GUIDE SEQUENCE</td>
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<tr>
<td>REPAIR PROTEIN</td>
<td></td>
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<tr>
<td>DONOR DNA</td>
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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="Image of RFP gene" /></td>
<td><img src="image2" alt="Image of guide sequence" /></td>
<td><strong>GFP DONOR DNA</strong></td>
</tr>
<tr>
<td><strong>Sickle cell disease</strong></td>
<td><img src="image3" alt="Image of hemoglobin gene with sickle cell mutation" /></td>
<td><img src="image2" alt="Image of guide sequence" /></td>
<td><strong>DONOR DNA</strong></td>
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CRISPR Components

- Sickle Cell Mutation
- Hemoglobin Gene
- Guide Sequence
- Cas9
- Donor DNA
- Repair Protein
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
Time Sequence Model: CRISPR Lab
**Completed Model**

**START**

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

**Cas9**

The guide sequence on Cas9 matches the RFP gene, allowing Cas9 to find the RFP gene.

**Cas9**

Cas9 then cuts the gene.

**END**

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

**Repair Proteins**

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

**GFP Gene**

Now the RFP gene has been replaced by the GFP gene in the bacterial DNA.
Articles: Ethics of CRISPR

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

Image credits: Patient: tiverylucky/Shutterstock.com; Rice field: Soichiro/Shutterstock.com
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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?
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?
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?
Contact Information

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    - https://sepuplhs.org
    - Twitter: @SEPUP_UCB

- Lab-Aids
  - Our fabulous publisher! Handles all sales, kits, etc.
    - https://www.lab-aids.com
  - Or go to the great staff at the back of the room. 😊