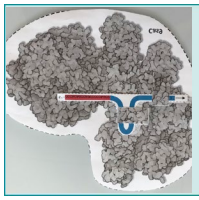

Building a Paper Model of CRISPR-Cas9

INTRODUCTION

"Get Ready for Genetically Engineered 'Super Chocolate'" (*Daily Mail*, May 10, 2018)

"Can the Gene-Editing Technology CRISPR Cure This Debilitating Brain Disease?" (*Newsweek*, April 10, 2018)

"CRISPR/Cas9 Silences Gene Associated with High Cholesterol" (*ScienceDaily*, April 26, 2018)

"Could Gene Editing Turn You into Captain America?" (*Vanity Fair*, August 30, 2019)

As these headlines show, there is much excitement about CRISPR. But what exactly is it?

CRISPR-Cas9 (often shortened to "CRISPR") is a biotechnology tool that can be used to edit the DNA in cells and organisms relatively cheaply and quickly. Since it was first described in 2012, the CRISPR-Cas9 system has generated much interest for its exciting potential to treat genetic diseases, defeat viruses, produce better crops, and even bring us "super chocolate." It has also raised concerns about food and patient safety, as well as the possible creation of designer babies or superhumans.

In this activity, you will explore how CRISPR-Cas9 works to edit DNA by building your own paper model of the system. You will use the paper model to learn how CRISPR-Cas9 is used to inactivate ("knock out") genes and to edit the sequence of genes in a more specific way. You will then explore an online model of CRISPR-Cas9 and see how this tool is being used in current research.

MATERIALS

- copies of the paper model sheets
- scissors
- clear tape

PART 1: A Paper Model of CRISPR-CAS9

In this part of the activity, you will use the materials provided to build a paper model of the CRISPR-Cas9 system and examine how it works. This model includes the following components:

- **Cas9:** a DNA-cutting enzyme called a nuclease
- **Guide RNA:** an RNA molecule that binds to Cas9 and allows it to find the target gene
- **Target DNA:** a DNA molecule that contains a "target gene" for CRISPR-Cas9 to cut
- **Random nucleotides:** nucleotides that can be inserted where the target gene is cut
- **Donor DNA:** DNA that can be used to edit the target gene in a more specific way

Building the Model

First, you will prepare the paper components to model the CRISPR-Cas9 tool.

1. Cut out the Cas9 enzyme and the two tabs from the sheet shown in Figure 1.

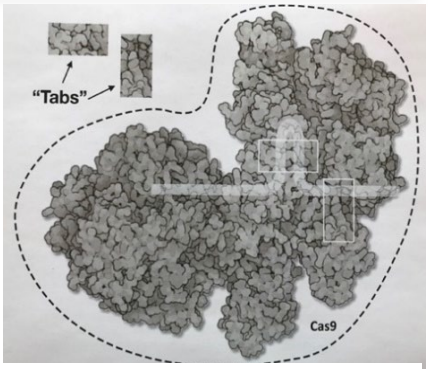

Figure 1. The Cas9 model sheet.

2. Place the two tabs over the rectangles outlined in white on the Cas9 enzyme. Tape down the **short** edges of each tab, making sure *not* to put tape over the long edges. The blue outlines in Figure 2 show where the tape pieces should go.

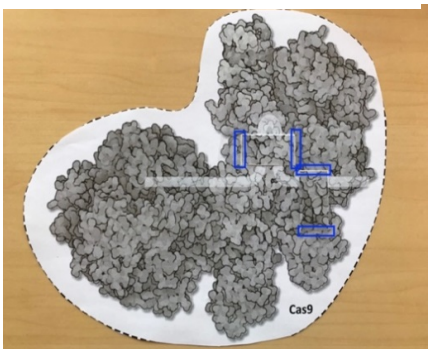

Figure 2. The Cas9 model with the tabs placed in the appropriate locations and taped down. The blue outlines show the locations of the tape. Note that the tape covers only the short ends of the tabs.

3. Cut out the two target DNA molecules, the two guide RNA molecules, the donor DNA, and the random nucleotide pieces from the sheet shown in Figure 3.

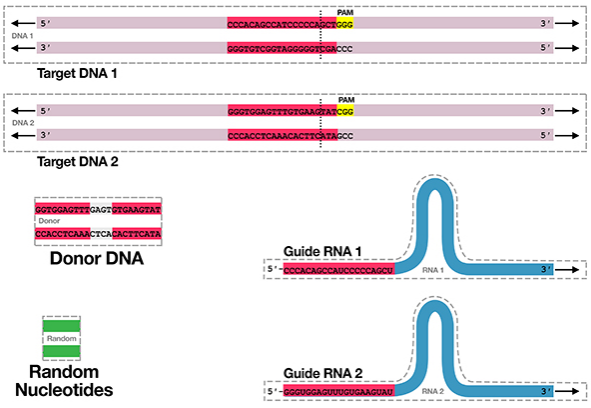

Figure 3. The RNA and DNA model sheet.

4. To build the CRISPR-Cas9 model, you will need to model binding the guide RNA to Cas9. Attach guide RNA 1 to the Cas9 enzyme by sliding it under the tabs, as shown in Figure 4.

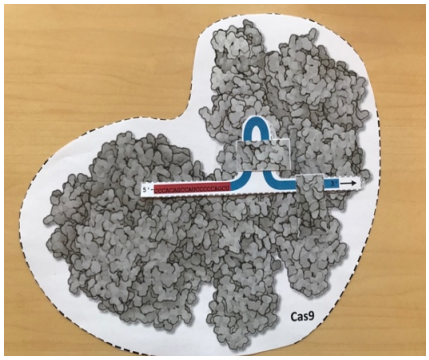

Figure 4. Model of CRISPR-Cas9 using guide RNA 1. The loop on the guide RNA should slide under the top tab, and the 3' end of the RNA should slide under the tab on the right.

In this model, the **blue** section of the guide RNA represents the part that binds to the Cas9 enzyme. The **red** section of the RNA, which has a sequence written out, represents a "targeting sequence" that is free to bind to a complementary DNA sequence.

Modeling Targeting and Binding

Your Cas9-RNA complex is now "programmed" to seek out a target DNA. Cas9 first recognizes and binds to a three-nucleotide sequence called PAM, which occurs throughout the genome. An example of a PAM sequence is highlighted in yellow on the target DNA in your model.

Once Cas9 binds to a PAM sequence, it unwinds the DNA. If the guide RNA matches the DNA sequence next to the PAM, the guide RNA will bind to the complementary DNA strand. If not, the DNA will zip back together and

Cas9 will keep binding to other PAM sequences until it finds the matching target DNA.

You will now model how the guide RNA finds its matching target DNA using **target DNA 1**. The target DNA 1 sequence is from a real gene called *MC1R*. This gene codes for a protein that affects the color of skin and hair.

5. Slide target DNA 1 under guide RNA 1, through the rightmost tab on the Cas9 enzyme. Line up the **red** targeting sequence on the guide RNA with the complementary sequence on the target DNA, as shown in Figure 5.

   Question 1a. Write down the guide RNA 1 sequence that binds to the DNA, and the complementary DNA 1 sequence that it binds to. Label the 5' and 3' ends of both strands.

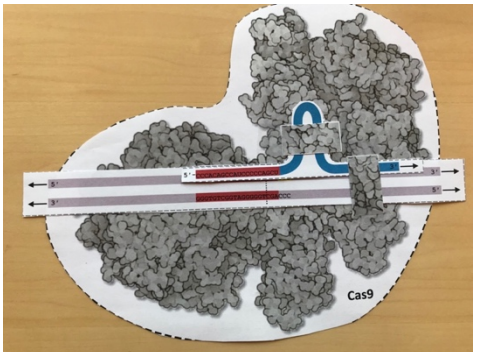

Figure 5. Model of the Cas9-guide RNA complex bound to target DNA 1.

Modeling Cleaving

Once the guide RNA binds to the DNA, it activates the nuclease activity (DNA-cutting ability) of the Cas9 enzyme. Cutting DNA is also called "cleaving." Cas9 always cleaves *both* strands of the DNA three nucleotides upstream (toward the 5' end) of the PAM sequence.

6. To model how Cas9 cleaves the DNA, use scissors to cut target DNA 1 along the dotted line, as shown in Figure 6. Do not cut the guide RNA.

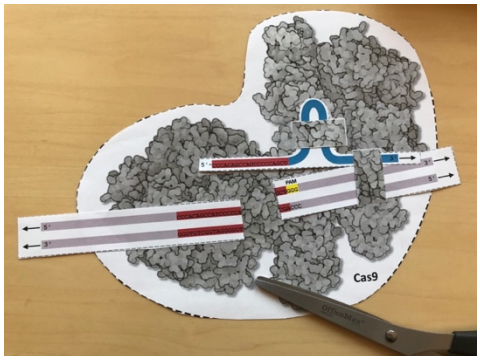

Figure 6. Model of Cas9 cleaving target DNA 1.

Modeling DNA Repair

After Cas9 cleaves the DNA, cellular enzymes will attempt to repair the break. CRISPR-Cas9 takes advantage of these repair mechanisms to alter the target gene sequence. You will now explore two applications of CRISPR-Cas9 that use different repair mechanisms.

First, you will model how CRISPR-Cas9 can be used to *inactivate* a target gene ("gene knockout"). In this case, the cell uses **nonhomologous end joining (NHEJ)**, a repair mechanism that is sometimes error-prone, to repair the DNA break.

7. To model a possible outcome of NHEJ when CRISPR-Cas9 is used, tape the cut pieces of target DNA 1 together with the "Random Nucleotides" piece between them. These random nucleotides represent a mutation that is likely to inactivate the target gene.

   Question 1b. Compare the sequences in the model you just made to your answer to Question 1a. How did the sequence of the gene change due to CRISPR-Cas9? Where was this change made (to the RNA, to one DNA strand, or to both DNA strands)?

   Question 1c. How might this change inactivate, or "knock out," a gene?

Next, you will model how CRISPR-Cas9 can be used to *edit* a target gene. In this case, the cell uses **homology-directed repair (HDR)**, which is less error-prone than NHEJ, to repair the DNA break. HDR fixes DNA using a template sequence, usually from a homologous chromosome. Scientists can provide a "donor DNA" as a template to "trick" the cell into using HDR. This approach can be used to replace a mutation with a normal, wild-type sequence or to add a new gene sequence ("gene knock-in").

8. Remove guide RNA 1 and target DNA 1 from your Cas9 enzyme, then repeat Steps 4–6 above using guide RNA 2 and target DNA 2. The target DNA 2 sequence is from a real mutant version of a gene called *MYBPC3*, which has a deletion of four nucleotides (GAGT) compared to the wild-type (functioning) gene. This deletion causes a type of heart disease.

   Question 2a. Write down the guide RNA 2 sequence that binds to the DNA, and the complementary DNA 2 sequence that it binds to. Label the 5' and 3' ends of both strands.

9. To model the outcome of HDR, place the "Donor DNA" piece over the cut pieces of target DNA 2 with the matching sequences overlapping. Tape the donor DNA down over the target DNA pieces so that they are all attached.

   Question 2b. Compare the sequences in the model you just made to your answer to Question 2a. How did the sequence of the mutant *MYBPC3* gene change due to CRISPR-Cas9?

   Question 2c. How might this change affect the mutant *MYBPC3* gene?

Additional Questions

3. Briefly describe a situation in which a scientist would want to "knock out" a gene.

4. Briefly describe a situation in which a scientist would want to edit a sequence or add a new sequence for a gene ("knock in" a gene).

5. CRISPR-Cas9 has been described as DNA scissors with a programmable GPS, or homing device. Use what you've learned from your model to explain this analogy.

PART 2: CRISPR Interactive Exploration

Launch the *CRISPR-Cas9 Mechanism & Applications* Click & Learn and select "How It Works." Scroll down through the steps to watch the animation, clicking on the buttons that appear to learn more.

1. Using the information in the Click & Learn, summarize each step of CRISPR-Cas9 in the table below.

Step	Summary

Think about the two-dimensional paper model you built in Part 1 and the three-dimensional model you saw in the Click & Learn, then answer the following questions.

2. What is one limitation of the paper model compared to the Click & Learn model?

3. What is one limitation of the Click & Learn model compared to the paper model?

4. What is one limitation of both models compared to studying the process in an actual cell?

PART 3: Extension to the Interactive Model Exploration (Optional)

Return to the *CRISPR-Cas9 Mechanism & Applications* Click & Learn and select "How It's Used." Watch the opening video, "A Breakthrough Technology," then scroll through the remaining videos on the right side of the screen.

1. Based on the video titles, select a scientist you want to study. Check the box next to your choice below.

   ☐ Jennifer Doudna

   ☐ David Liu

   ☐ Robert Reed

   ☐ Neville Sanjana

   ☐ Amy Wagers

2. View the videos associated with the scientist you selected. Summarize each video in one sentence in the table below.

Video Title	Video Summary

After viewing the videos, reflect on everything you have learned about the CRISPR-Cas9 biotechnology tool and answer the questions below.

3. What are **three** things that you learned?

4. What are **two** things you found particularly interesting?

5. What is **one** question you still have about CRISPR-Cas9?