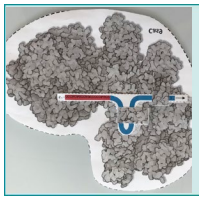


Building a Paper Model of CRISPR-Cas9

hhmi | BioInteractive

Activity
Educator Materials

OVERVIEW

CRISPR-Cas9, commonly referred to as just CRISPR, is often mentioned in the news for its potential to treat genetic diseases. In this activity, students explore and learn about this biotechnology tool by building a two-dimensional paper model of the CRISPR system. They then apply their knowledge of complementary base pairing to model how CRISPR targets specific DNA sequences. Students are asked to compare their paper model to an online three-dimensional model. In an optional extension, students watch and reflect on videos of scientists explaining how they use CRISPR in their research.

Additional information related to pedagogy and implementation can be found on this resource's webpage, including suggested audience, estimated time, and curriculum connections.

KEY CONCEPTS

- CRISPR-Cas9 can be used to target specific genes in an organism's genome.
- CRISPR-Cas9 can be designed to either inactivate ("knock out") genes or to edit genes.
- CRISPR-Cas9 is a versatile biotechnology tool that can be easily modified to meet the needs of a research project.

STUDENT LEARNING TARGETS

- Apply knowledge of DNA structure, function, and base pairing to describe how CRISPR-Cas9 can be used to inactivate and edit genes.
- Compare two different models of a biological process.
- Explore different types of research projects that apply CRISPR-Cas9 technology (extension).

PRIOR KNOWLEDGE

Students should:

- be familiar with the roles of DNA and RNA in the storage, expression, and transmission of genetic information
- have a basic understanding of the processes of DNA replication, as the CRISPR-Cas9 technology involves many similar steps, such as DNA cleaving and base pairing

MATERIALS

- access to the *CRISPR-Cas9 Mechanism & Applications* Click & Learn
- copies of the "Student Handout"
- copies of "Cas9" and "RNA and DNA" model sheets (or precut/preassembled models)
- scissors
- clear tape

BACKGROUND

CRISPR-Cas9 (often shortened to "CRISPR") is a technology that allows scientists to edit a cell's DNA. Since it was first described in 2012, CRISPR has generated much interest both for its exciting potential for treating genetic diseases as well as for potential ethical and safety concerns, such as creating designer babies and superhumans. The CRISPR system was first discovered in bacteria, where it functions as a type of immune system. Scientists have modified the bacterial system to produce a biotechnology tool for editing DNA.

CRISPR-based technologies are widely used in research and have been applied in a broad range of biological studies. Treatments using CRISPR technology are also being developed for several genetic diseases, including sickle cell disease and cystic fibrosis. The technology is relatively cheap, easy to use, and allows researchers to ask new questions and get results faster than previously possible.

BioInteractive's *CRISPR-Cas9 Mechanism & Applications* Click & Learn provides more information on how CRISPR works and how it is used by scientists. The *Central Dogma and Genetic Medicine* Click & Learn explores how CRISPR and other technologies can be used to treat genetic diseases. To view actual microscopy images of the CRISPR-Cas9 system at work in a cell, see Shibata et al. (2017), an open-access article from *Nature.*

The paper model in this activity, which is an adaptation of one developed by Dr. David Wollert at Chattanooga State Community College, shows some of the key components and processes in the CRISPR system. The activity explores two different applications of CRISPR, gene inactivation ("knockout") and gene editing, and how they make use of the cell's processes for repairing double-stranded DNA breaks.

- Gene inactivation with CRISPR makes use of **nonhomologous end joining (NHEJ)**, which is the cell's main repair process for fixing double-stranded DNA breaks. Students may wonder how CRISPR can cause mutations using this process. During NHEJ, the broken ends of the DNA are brought together and rejoined. This process can be error-prone, because sometimes nucleotides are lost from the broken ends and re-added incorrectly by the cell's repair machinery. If the DNA sequence is repaired correctly by NHEJ, Cas9 will just bind to the sequence using the guide RNA and cut the DNA again. Although the cell can keep repairing the DNA, Cas9 will continue cutting it until the cell finally adds the wrong nucleotides, often causing the gene to lose function. Once the DNA sequence has the wrong nucleotides, Cas9 will no longer cut it again, because the guide RNA will no longer match and bind to the DNA.
- Gene editing with CRISPR makes use of **homology-directed repair (HDR)**, a repair process that cells typically use only shortly after DNA replication, in the S and G2 phases, when sister chromatids are present and can serve as templates for repairing the breaks. Since HDR repairs breaks by copying from a homologous template, it is more accurate than NHEJ. When using the CRISPR-Cas9 technology, scientists supply the homologous template in the form of "donor DNA," so that the cell will use HDR, rather than NHEJ, to repair the double-stranded DNA break. Scientists can design the homologous template to include a new sequence or correct an existing sequence in a cell.

This activity shows how two specific DNA sequences could be targeted using CRISPR-Cas9 technology. Both of these target sequences are from real genes:

- The target DNA 1 sequence is from the human *MC1R* gene. This gene is associated with melanin production in skin and hair. In this activity, CRISPR-Cas9 is used to inactivate the gene, which would be expected to cause a change in coloration.
- The target DNA 2 sequence is from a gene called *MYBPC3*. A deletion of four bases (GAGT) in this gene causes a genetic disease called hypertrophic cardiomyopathy. This disease occurs in 1 out of every 500 births, making it one of the most common genetic heart conditions. It can result in heart attacks in adults and is a common cause of sudden death in otherwise healthy young athletes. As modeled in this activity, CRISPR-Cas9 has been used to edit the mutant *MYBPC3* gene in human embryonic cells by inserting the missing GAGT sequence (Ma et al. 2017).

Note that the paper model makes certain simplifications in its representation of CRISPR. For example:

- Actual guide RNAs are much longer than what is shown in the paper model. In reality, each guide RNA forms a complex three-dimensional shape containing several double-stranded loops and twists. This three-dimensional shape allows the guide RNA to bind to the nuclease Cas9, similar to how a substrate binds to an enzyme. For more information on how RNA is able to form three-dimensional structures, watch the *RNA Folding* video clip featuring Nobel laureate Thomas Cech.
- In the cell, Cas9 binds to PAM sequences, which occur throughout the genome. When it binds PAM, Cas9 unwinds the double-stranded DNA. This step is not shown in the paper model. If the guide RNA matches the target DNA, it will bind to one of the two DNA strands by complementary base pairing to form a DNA-RNA helix. This step is also not shown in the model.
- DNA repair pathways are more complex than they appear in this model. In the part of the model involving homology-directed repair, for example, it may look as if the donor DNA is spliced directly into the target DNA and replaces parts of the target sequence. In reality, the repair involves homologous recombination between the donor and target DNA.

TEACHING TIPS

- In addition to the *CRISPR-Cas9 Mechanism & Applications* Click & Learn, this activity can be paired with several other BioInteractive resources involving CRISPR:
  - The "Using CRISPR to Identify the Functions of Butterfly Genes" activity explores how scientists have used CRISPR to knock out genes that affect the colors and patterns on butterfly wings. Students design their own guide RNA to inactivate a butterfly gene and examine the resulting phenotype. This research is also discussed in Robert Reed's videos in the "How It's Used" tab of the CRISPR Click & Learn.
  - The "Winging It: Analyzing a Scientific Paper" activity has students analyze parts of a scientific paper (Zhang et. al. 2017) that presents the research introduced in the "Butterfly Genes" activity above. The "Winging It" activity is most appropriate for an undergraduate audience.
  - The *Central Dogma and Genetic Medicine* Click & Learn, its accompanying worksheet, and the *Genes as Medicine* short film show how CRISPR and other biotechnology tools can be used to treat genetic diseases.
- Building the model involves cutting several small shapes. If some students find this task challenging, structure the activity so that you are providing different ways for students to access the models. It may be helpful to provide students with precut shapes or preassembled models, or to group students so that they can divide the task among themselves.
- To use the models for multiple sections or for multiple years, you may cut out and laminate the model pieces. Ensure that the pieces work together after lamination.
- Make sure that the opening beneath the rightmost tab of the model is wide enough for both the RNA and DNA pieces to slide through. Avoid putting tape over the long edges of the tab, or else it might be difficult to slide the RNA and DNA pieces through.
- Students should look for exact matches between the guide RNA and target DNA, with U (uracil) in the RNA matching with T (thymine) in the DNA.
- In the "Student Handout" for this activity, students build the paper model (Part 1) before investigating the online model in the CRISPR Click & Learn (Part 2). The activity would work equally well with the order of these parts flipped. To do so, have students complete the table in Part 2 before doing the paper model in Part 1. They can then return to the Click & Learn as needed to answer the comparison questions at the end of Part 2 and to complete the extension.

- If you feel it is appropriate for your class, consider moderating a discussion regarding the ethics of gene editing. Editing of the *MYBPC3* gene, which is shown in target DNA 2, can be used as a starting point for this discussion. Scientists have edited the *MYBPC3* gene in human oocytes, which were fertilized and allowed to develop to the eight-cell and blastocyst stages (Ma et al. 2017).

ANSWER KEY

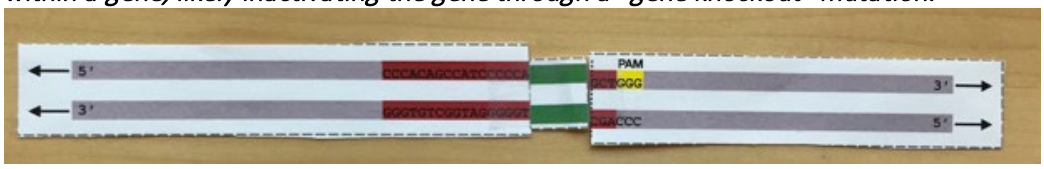
PART 1: A Paper Model of CRISPR-CAS9

1. Questions about target DNA 1:
   a. 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.
   RNA: 5'-CCCACAGCCAUCCCCCAGCU-3'
   DNA: 3'-GGGTGTCGGTAGGGGGTCGA-5'

   b. 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)?
   Random nucleotides were inserted into the gene's sequence. Students may also show the specific sequence where the nucleotides were integrated. The change was made to both strands of DNA.

   c. How might this change inactivate, or "knock out," a gene?
   These changes can inactivate a gene by preventing it from producing a functional protein. For example, random nucleotides in the gene's sequence may make it code for the wrong amino acids, resulting in a nonfunctional protein. (It's possible that the protein will still function if the resulting mutation is a silent mutation. This may be the case if three nucleotides are inserted precisely at the break, resulting in an in-frame mutation.)

The final model for target DNA 1 (NHEJ repair) is shown below. This model shows random nucleotides inserted within a gene, likely inactivating the gene through a "gene knockout" mutation.



2. Questions about target DNA 2:
   a. 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.
   RNA: 5'-CCCACCUCAAACACUUCAUA-3'
   DNA: 3'-GGGTGGAGTTTGTGAAGTAT-5'

   b. 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?
   Four extra nucleotides (GAGT) were inserted into the gene's sequence, which could make it match the wild-type (functioning) gene. The rest of the sequence did not change.

   c. How might this change affect the mutant *MYBPC3* gene?
   It could correct the mutant gene sequence so that it produces a wild-type (functional) protein.

The final model for target DNA 2 (HDR with donor DNA) is shown below. The sequence is the same as before editing, with the exception of four additional nucleotides (GAGT). This addition corrects a deletion mutation in the mutant MYBPC3 *gene, forming a "gene knock-in."*

3. Briefly describe a situation in which a scientist would want to "knock out" a gene.
Student answers may vary. For example, knockout mutations could be used to determine the function of genes. Scientists might also want to knock out a gene that causes a disease, although they would need to be sure knocking it out does not cause other problems.

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).
Student answers may vary. For example, scientists might want to replace a mutated gene that causes a harmful phenotype, such as a disease, with a wild-type copy of the gene. They might also want to insert the sequence for a new protein or reporter gene, such as GFP.

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.
Cas9 is a nuclease, which means it cuts DNA similar to how scissors cut paper. When paired with a target RNA sequence, Cas9 cuts DNA only in specific places along the genome, ones with a matching DNA sequence. In this way, the DNA scissors "home in" on a target gene.

PART 2: CRISPR Interactive Exploration

1. Using the information in the Click & Learn, summarize each step of CRISPR-Cas9 in the table below.
Student summaries will vary. Examples are shown below.

Step	Summary
Targeting	*Cas9 randomly binds to a three-nucleotide sequence called PAM (5'-NGG-3', where the N represents any nucleotide) that is abundant throughout the human genome.*
Binding	*Cas9 unwinds the DNA helix upstream of PAM. If the DNA sequence matches the guide RNA sequence, then the RNA and complementary DNA will bind to form a DNA-RNA double helix.*
Cleaving	*Cas9's nuclease activity is activated. It cuts both strands of the DNA three nucleotides upstream from PAM.*
DNA Repair	*Repair enzymes fix the break in the DNA. If a homologous template is NOT present, the cell repairs the break using nonhomologous end joining (NHEJ), a more error-prone repair process that may eventually lead to a mutation that inactivates the gene. If a homologous template is available, the cell repairs the break using homology-directed repair (HDR), which can be manipulated to change the target DNA sequence.*

2. What is one limitation of the paper model compared to the Click & Learn model?
Student answers will vary. Examples: The paper model is two-dimensional and less detailed, it doesn't model the formation of the DNA-RNA helix, it doesn't reflect the real structure of the guide RNA, etc.

3. What is one limitation of the Click & Learn model compared to the paper model?
Student answers will vary. Examples: The Click & Learn model does not show actual sequences (so you cannot see complementary base pairing), it is a set animation that can't be directly manipulated, etc.

4. What is one limitation of both models compared to studying the process in an actual cell?
Student answers will vary. Examples: Neither model includes the other proteins in the cell, neither shows how DNA repair actually works, neither reflects the actual timescale of the process, etc.

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

Student answers will vary depending on the scientist/videos selected. You may wish to address responses to the last question ("What is one question you still have about CRISPR-Cas9?") in class.

REFERENCE

Ma, Hong, Nuria Marti-Gutierrez, Sang-Wook Park, Jun Wu, Yeonmi Lee, Keiichiro Suzuki, Amy Koski, et al. "Correction of a pathogenic gene mutation in human embryos." *Nature* 548, 7668 (2017): 413–419. https://doi.org/10.1038/nature23305.

CREDITS

Written by Ann Brokaw, Rocky River High School, OH

CRISPR paper model originally developed by David Wollert, Chattanooga State Community College, TN

Edited by Esther Shyu, Laura Bonetta, HHMI
Scientific review by Vincent Buonaccorsi, Juniata College, PA