Using CRISPR to Identify the Functions of Butterfly Genes

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Activity
Educator Materials

OVERVIEW

CRISPR-Cas9, commonly referred to as just CRISPR, is a biotechnology tool that can inactivate, or "knock out," genes. In this activity, students explore using CRISPR-Cas9 to knock out butterfly genes in order to determine their functions. Students first learn how CRISPR-Cas9 identifies and alters a target sequence in DNA. They then design their own CRISPR-Cas9 system to inactivate a butterfly gene and examine the resulting phenotype. The activity includes an optional extension in which students apply what they have learned to determine the function of a different gene. This activity can be used to review concepts of sequence complementarity, genotype-to-phenotype connections, and mutations.

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 inactivate specific genes in an organism's genome.
- A single strand of RNA can bind to a single strand of DNA by sequence complementarity.
- Changes in the DNA sequence of an exon of a gene can affect the phenotype of an organism.

STUDENT LEARNING TARGETS

- Identify complementary nucleotide sequences to plan the design of a biotechnology tool.
- Interpret the connection between genotype and phenotype for a particular trait.

PRIOR KNOWLEDGE

Students should be familiar with:

- the process of gene expression
- the difference between introns and exons
- the connection between genotype and phenotype
- DNA base-pairing rules
- RNA structure

MATERIALS

- copies of the "Student Handout"
- access to the *CRISPR-Cas9 Mechanism & Applications* Click & Learn
- (optional) color versions of the "Butterfly Photos," which are enlarged versions of Figures 2–6 in the "Student Handout"

BACKGROUND

CRISPR-Cas9 (often shortened to "CRISPR") is a technology that allows scientists to edit a cell's DNA, which can be used to inactivate ("knock out") genes. Since it was first described in 2012, CRISPR has generated much interest for its exciting potential in both research and medicine. 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 have become widely used in research and have been applied in a broad range of biological studies. 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.

TEACHING TIPS

- The "How It Works" section of the *CRISPR-Cas9 Mechanism & Applications* Click & Learn covers much of the same information as Part 1 of this activity. It may be helpful for students to explore the Click & Learn first, then do Part 1 as a review.
- In addition to the CRISPR Click & Learn, this activity can be paired with several other BioInteractive resources involving CRISPR:
  - The "Building a Paper Model of CRISPR-Cas9" activity has students build and explore a two-dimensional paper model of the CRISPR system. This activity is appropriate for both high school and college audiences.
  - The "Winging It: Analyzing a Scientific Paper" activity has students analyze parts of the research article (Zhang et al. 2017) that is the source of Figures 2–4 in the "Student Handout." 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.
- To minimize the use of class time, students could do some of this activity as homework (for example, the Click & Learn question at the end of Part 3 or the optional extension section).
- The "Butterfly Photos" PDF contains enlarged versions of Figures 2–6 in the "Student Handout." These images should be shown to students in color. The images can be projected on a screen if color printing is not available. Another option is to share the images online.
- 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 want to know more about 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.

ANSWER KEY

PART 1: Using CRISPR-Cas9 to Inactivate Genes

1. With a partner, discuss what it means to inactivate a gene and how it could be done. Record your ideas.

   Inactivating a gene means preventing that gene from being expressed as a protein. Student ideas for how to inactivate a gene may vary. They may suggest changing the sequence of the gene so that it no longer produces a functional protein, or using a transcription repressor or RNA interference to prevent the protein from being made.

2. Can you think of additional ways to find out what genes do? Record one or two ideas.
   Answers will vary but could include ideas about analyzing the DNA sequence of the gene and looking for similar genes with known functions in other species. Another option is expressing the gene in bacteria to produce a protein and then studying the function of that protein.

3. The partial gene (DNA) sequence below contains multiple PAM sequences. Highlight **six PAM sequences** in the top (5′ to 3′) strand.

5'-GCACGGCGGAGCGGTTCTTGGCAGCGGCCGCACGATCTCGTTGCCGCCGG-**3'**
3'-CGTGCCGCCTCGCCAAGAACCGTCGCCGGCGTGCTAGAGCAACGGCGGCC-**5'**

4. Below is a partial sequence of a guide RNA. The underlined section of the RNA is designed to match a specific target DNA sequence.

5'-GGCGGAGCGGUUCUUGGCAGGUUUUAGAGCUAGAAAUAGC-**3'**

Review the partial gene sequence reshown below. It contains a target DNA sequence that matches the guide RNA above. Highlight the **one PAM sequence** in the top (5′ to 3′) strand that is *next to* this target DNA sequence.

5'-GCACGGCGGAGCGGTTCTTGGCAGCGGCCGCACGATCTCGTTGCCGCCGG-**3'**
3'-CGTGCCGCCTCGCCAAGAACCGTCGCCGGCGTGCTAGAGCAACGGCGGCC-**5'**

5. Write down the guide RNA sequence that binds to the DNA, and the DNA sequence that it binds to (the complement of the target DNA). Label the 5' and 3' ends for both the RNA and DNA strands.

   RNA: 5'-GGCGGAGCGGUUCUUGGCAG-3'

   DNA: 3'-CCGCCTCGCCAAGAACCGTC-5'

6. Rewrite the target DNA sequence and its complement below, indicating where Cas9 would cut both strands of DNA with a large space or vertical line (|).

   5'-GGCGGAGCGGTTCTTGG CAG-3'

   3'-CCGCCTCGCCAAGAACC GTC-5'

7. DNA repair errors include losing or inserting random nucleotides at the cut site. Explain how these changes might inactivate a gene.

   Random deletions or insertions can inactivate a gene by preventing it from producing a functional protein. For example, these changes may make the gene's sequence 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.)

PART 2: Inactivating Genes in Butterflies

1. What are exons and introns, and how do they differ?

   Exons are the parts of genes that code for proteins. They are transcribed into messenger RNA (mRNA) and then translated into the proteins. Introns are the parts of genes that do not code for proteins. They are removed from mRNA before translation through RNA splicing.

2. Figure 1 shows that the target DNA sequences are all in exons. Why might scientists want to target sequences in exons, rather than introns, when inactivating genes?

   Exons are the parts of the gene that will be translated into the protein. So changes in the DNA sequence of exons are more likely to affect the protein and thus the function of the gene.

PART 3: Designing a Guide RNA

1. Underline a 20-nucleotide **target DNA sequence** in the top (5′ to 3′) strand of the exon above. Remember that this sequence should be directly upstream (on the 5′ end) of a PAM sequence (5′-NGG-3′).

2. Highlight the **PAM sequence** that is next to your target DNA sequence. This is where Cas9 will bind.

   There are multiple correct answers for 1 and 2. One example is shown on the next page.

5'-CGACACCGGTTCCAGCGCTCGAGCCACGCGAAGCTTCAGGCGCTGTGGCTGGAAG
3'-GCTGTGGCCAAGGTCGCGAGCTCGGTGCGCTTCGAAGTCCGCGACACCGACCTTC

CGCACTACCAGGAAGCGGAGCGCCTCCGCGGTCGCCCGCTCGGGCCCGTCGACAA
GCGTGATGGTCCTTCGCCTCGCGGAGGCGCCAGCGGGCGAGCCCGGGCAGCTGTT

GTACCGGGTGCGGAAGAAGTTCCCTCTGCCGAGGACTATTTGGGACGGCGAACAG-**3'**
CATGGCCCACGCCTTCTTCAAGGGAGACGGCTCCTGATAAACCCTGCCGCTTGTC-**5'**

3. Rewrite the target DNA sequence and its complement below, indicating where Cas9 would cut both strands of DNA with a large space or vertical line (|).
   Answers will vary depending on the target DNA chosen. The answer using the target DNA above is:
   5'-CTCGAGCCACGCGAAGC TTC-3'
   3'-GAGCTCGGTGCGCTTCG AAG-5'

4. Record the 20-nucleotide **guide RNA sequence** that matches your target DNA sequence. This sequence should *not* include the PAM.
   Answers will vary depending on the target DNA chosen. The answer using the target DNA above is:
   5'-CUCGAGCCACGCGAAGCUUC-3'

5. Robert Reed's lab at Cornell University designed guide RNAs to knock out the *optix* gene in three species of butterflies: the painted lady (*V. cardui*), the common buckeye (*Junonia coenia*), and the Gulf fritillary (*Agraulis vanillae*). Figures 2–4 compare the wings of the wild-type (control) butterflies to those of *optix* knockouts (butterflies that had their *optix* gene inactivated).
   a. For each species shown, describe how the wings of the *optix* knockout compare to those of the wild-type butterfly.
      Answers will vary based on student observations. Possible observations include that the optix *knockout lost the orange color, gained a blue color, or lost some distinct stripes and patterning.*
   b. Based on the figures, predict the function of the *optix* gene. Does the gene have the same function in all three butterfly species? If not, explain how it may differ among the species.
      Answers will vary based on student observations. Students may say that the function of the optix *gene in all three species appears to be similar, and that this function is related to the colors and patterns on the butterflies' wings. In two species, the main effect of knocking out* optix *seems to be the loss of orange. In the third species, knocking out* optix *seems to make the wings more blue.*
   c. Justify your prediction with evidence from all three figures. Be specific.
      Answers will vary based on student observations. In V. cardui, *the wild-type butterfly has many orange regions on its wings, whereas the* optix *knockout mostly lacks orange. In* A. vanillae, *the orange wings of the wild-type butterfly have turned black in the knockout. In* J. coenia, *the knockout's wings also lack orange-colored regions and show regions of blue instead.*

6. Launch the *CRISPR-Cas9: Mechanism & Applications* Click & Learn and select "How It's Used." Scroll through the videos on the right side of the screen and watch the three video interviews with Robert Reed. Revise your answers to Question 5 based on information from the videos.
   Student revisions will vary. The videos mention that knocking out optix *causes changes in color, and that these changes differ depending on the butterfly species.*

EXTENSION: Determine the Function of a Different Gene

1. A partial sequence of an exon from the butterfly's *spalt* gene is shown below. This time, only one of the DNA strands is shown.
   a. Underline a 20-nucleotide **target DNA sequence** in the exon above. Remember that this sequence should be directly upstream of a PAM sequence.

b. Highlight the **PAM sequence** that is next to your target DNA sequence.

There are multiple correct answers for a and b. One example is shown below.

5'CGATATCTGGACCAATTTCAATAGCAACAGGACTACGTACTTTTCCTTCATATCCACTATTTCCAAA
TTCCCCACCAAGCAGTGTCTCATCTGGATGTCTTACACCTTTCCAAAGTAATCCCAACAGCATAATAG
ACAGTGACATAACTCGTGATCCCATATTTTATAATTCACTTTTACCGCGTCCTGGAAGTAATGACAAC
TCTTGGGAAAGTTTGATTGAAATTACTAAAACTTCAGAAACGTCAAAATTGCAACAGTTAGTAGATA
ATATTGATAACAAAGTTACTGATCCTAACGAGTGTATTGTATGTCATCGCGTCTTATCTTGTAAAAGT
GCTTTACAGATGCACTACCGAACTCATACCGGGGAAAGACCTTTCAGATGTAAATTGTGCGGTCGTG
CTTTTACTACAAAGGGCAATTTAAAAACTCATATGGGTGTCCATCG**-3'**

c. Rewrite the target DNA sequence, indicating where Cas9 would cut the DNA with a large space or vertical line (|).

Answers will vary depending on the target DNA chosen. The answer using the target DNA above is:

5'-ATGCACTACCGAACTCA TAC-3'

2. Record the 20-nucleotide **guide RNA sequence** that matches your target DNA sequence.

Answers will vary depending on the target DNA chosen. The answer using the target DNA above is:
5'-AUGCACUACCGAACUCAUAC-3'

3. The Reed lab used CRISPR-Cas9 to knock out the *spalt* gene in two species of butterflies: *V. cardui* and *J. coenia*. Figures 5–6 compare the wings of the wild-type butterflies to those of *spalt* knockouts.

a. For each species shown, describe how the wings of the *spalt* knockout compare to those of the wild-type butterfly.

Answers will vary based on student observations. Possible observations include that the spalt *knockout has lost, reduced, and/or simplified wing spots, or altered spot or stripe patterns.*

b. Based on the figures, predict the function of the *spalt* gene. Does the gene have the same function in both butterfly species? If not, explain how it may differ between the species.

Answers will vary based on student observations. Students may say that the function of the spalt *gene in both species appears to be similar, and that it is involved in the formation of wing spots.*

c. Justify your prediction with evidence from both figures. Be specific.

Answers will vary based on student observations. In J. coenia*, the wings of the* spalt *knockout show missing or reduced spots. In* V. cardui*, the knockout's wings have less-defined spots, with some circles and colors missing.*

REFERENCES

Zhang, Linlin, Anyi Mazo-Vargas, and Robert D. Reed. "Single master regulatory gene coordinates the evolution and development of butterfly color and iridescence." *Proceedings of the National Academy of Sciences* 114, 40 (2017): 10707–10712. https://doi.org/10.1073/pnas.1709058114.

Zhang, Linlin and Robert D. Reed. "Genome editing in butterflies reveals that *spalt* promotes and *Distal-less* represses eyespot colour patterns." *Nature Communications* 7, 1 (2016): 11769. https://doi.org/10.1038/ncomms11769.

CREDITS

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