Extension: CRISPR/Cas paper model
Using CRISPR/Cas to knock out genes in snails

A. What is the Cas9 PAM sequence?

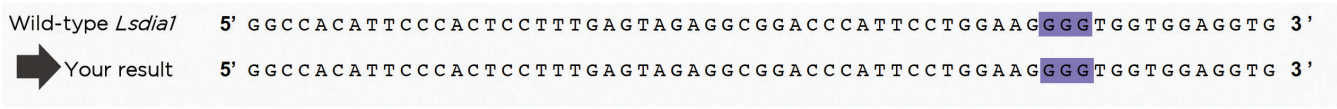
5' N G G 3'

Where N can be any base

B. How does the requirement that Cas9 bind to a PAM sequence affect the ability of scientists to target Cas9 to the exact DNA sequence they are interested in?

Cas9 can only be targeted to regions of the genome adjacent to a PAM sequence. DNA sequences that do not contain a PAM sequence would be inaccessible to Cas9-mediated gene editing.
Because NGG occurs so commonly, it is relatively easy to find a PAM sequence in any region of the DNA that a scientist is interested in.

C. Compare the repaired DNA sequence from your paper model to wild-type sequence. On the "your result" sequence below (indicated by the arrow), cross out the DNA bases that were deleted when you repaired the DNA.



This answer depends on what the students chose to do in Step 7 where they model NHEJ. There are 36 possible sequences given the parameters that students can chose to delete between 0-5 bases on either side of the cut site.

D. Is your resulting sequence the same as any of the mutant sequences that scientists found after using this guide RNA?

This answer depends on what the student chose to do in Step 7, where they model NHEJ. Given the number of possible outcomes, students sequences are likely to be different from those obtained by scientists, but may match.

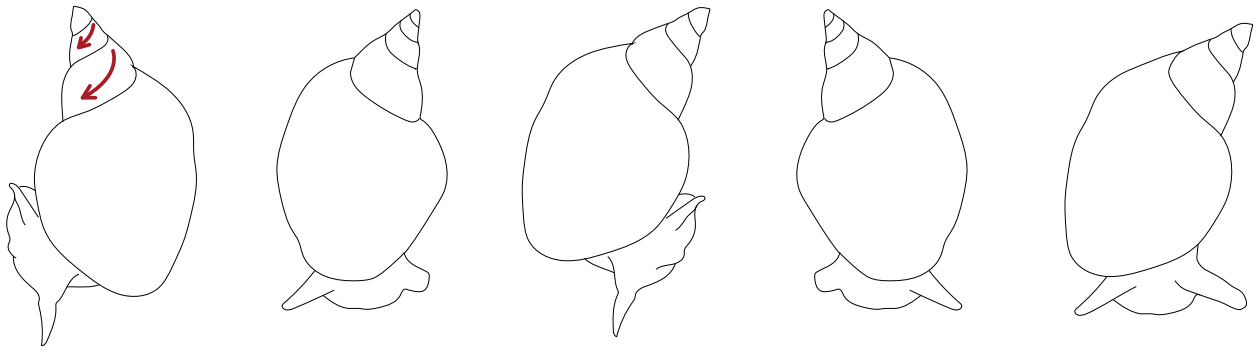
E. Compare your mutation to those made by other students. Did any groups create the same mutants? Using just this one guide RNA, how many different mutations were created?

It is likely that groups will have different mutants. This answer also depends on what the students chose to do in Step 7 where they model NHEJ. There are 36 possible sequences given the parameters that students can chose to delete between 0-5 bases on either side of the cut site.

F. NHEJ introduces random mutations. Considering this, why do you think it is important for scientists to sequence the cell's DNA after CRISPR/Cas genome editing?

Scientists need to sequence the cell's DNA after CRISPR/Cas genome editing to determine the specific mutations introduced after NHEJ. It is also possible that Cas9 did not cut the target DNA or for the DNA to be repaired without the introduction of mutations.

G. You also breed these genetically modified snails to generate offspring that are homozygous for each mutation. Below are sketches of these homozygous mutants (Abe and Kuroda, 2019).



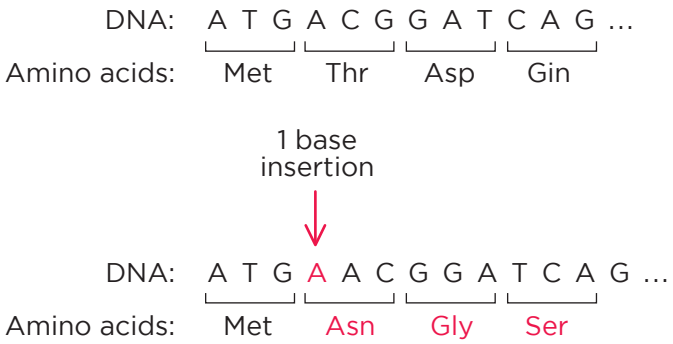
Wild-type *Lsdia1* **Shell coils clockwise** | **Mutant 1** | **Mutant 2** | **Mutant 3** | **Mutant 4**

Use the DNA sequences and the images of the homozygous snails to characterize each mutation in the table below

Mutant	Mutation type (insertion or deletion)	# of bases changed	Shell coils clockwise or counterclockwise?
1	Deletion	13	Counterclockwise
2	Deletion	4	Counterclockwise
3	Deletion	21	Clockwise
4	Insertion	1	Counterclockwise

H. In the snails above, the DNA that was altered will still be transcribed into mRNA, and that mRNA will still be translated by the ribosome. The sequence observed in Mutant 3 is the most severe mutation in terms of the total number of bases changed compared to the wild-type *Lsdia1* sequence. However, this mutation does not affect the direction in which the snail's shell spirals. Can you think of a reason why this might be the case? Hint: think about the number of bases deleted and how the instructions for making protein are encoded in DNA.

The information in DNA is read three bases at a time in units called codons, and each codon specifies a particular amino acid. When 1 or 2 DNA bases are added or removed, it shifts the way the codons are read, such that all the information after the mutation is read incorrectly. Scientists refer to this as a frameshift mutation. Frameshift mutations drastically effect the protein being made since every amino acid after the mutation will likely be affected.



On the other hand, if DNA bases are added or removed in multiples of three, the amino acids after the mutation remain the same. In mutant 3, 21 bases were deleted, which means 7 amino acids are missing from the protein, but the rest of the amino acid sequence remains unchanged. While it depends on the specific protein and the particular amino acids lost, it is possible for this to have a negligible effect on protein function.

I. Remember that the reason scientists wanted to knock out the Lsdia1 gene in snails was to test their hypothesis that the Lsdia1 gene determined the direction in which a snails shell spiraled. Does the experimental evidence support this hypothesis? Explain your reasoning.

The snails' parents had shells that spiraled clockwise, so the experimental snails would also be expected to have shells that spiraled clockwise. But after the *Lsdia1* gene was disabled, the snails' shells spiraled counterclockwise. This suggests that the *Lsdia1* gene does in fact control the direction in which a snail's shell spirals and supports the scientists' hypothesis.

J. DNA is said to have a universal genetic code. Cas9 is a bacterial protein. Explain why a universal genetic code allows us to use any organism, including snails, to express the *cas9* gene as protein.

Because the cells of all organisms transcribe and translate genetic information in the same way, theoretically, any organism can produce any protein from any other organism if they are provided with the DNA instructions.

K. Compare your guide RNA sequence with your classmates'. How many different guide RNAs did your class design to disrupt just this short region of the *Lsdia1* gene?

This answer depends on which PAM site the students chose to use in Step 9 where they design a guide RNA. Based on the section of the target DNA used in the model, there are 14 possible PAM sites that could be used to disrupt the *Lsdia1* gene.

L. Why is it helpful for scientists that the Cas9 PAM sequence is relatively common in the genome?

Because the guide RNA must be adjacent to a PAM, having a common PAM sequence means that there are typically numerous potential guide RNA sequences that can be used to disrupt even a short target DNA sequence.

Extension: CRISPR/Cas paper model
Sickle cell gene therapy

Reading questions

1. Sickle cell disease is characterized by the presence of sickle shaped red blood cells. According to the text, what health problems can sickle shaped red blood cells cause?

Sickled red blood cells get stuck in capillaries. The location of these blockages determines the symptoms and severity, but pain, infection, or death are all possible.

2. Which globin subunits make up adult hemoglobin protein? Select all that apply.
   a. α-globin
   b. β-globin
   c. γ-globin

3. Which globin subunits make up fetal hemoglobin protein? Select all that apply.
   a. α-globin
   b. β-globin
   c. γ-globin

4. Which gene is mutated in sickle cell disease?
   a. α-*globin*
   b. β-*globin*
   c. γ-*globin*

5. What protein switches off the production of fetal hemoglobin after birth?

BCL11A

6. Complete the following sentences by circling the correct term in parentheses:
   When BCL11A protein production is high, then fetal hemoglobin production is **(high/low)**.
   When BCL11A protein production is low, then fetal hemoglobin production is **(high/low)**.
   Therefore, to increase fetal hemoglobin production after birth, scientists want to **(increase/decrease)** the production of BCL11A protein in red blood cells.

7. Which DNA sequence is being targeted for genome editing in the first clinical trial using CRISPR/Cas to cure sickle cell disease?
   a. β-*globin* gene
   b. γ-*globin* gene
   c. *BCL11A* regulatory DNA

Paper model questions

A. What is the Cas9 PAM sequence?

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B. How does the requirement that Cas9 bind to a PAM sequence affect the ability of scientists to target Cas9 to the exact DNA sequence they are interested in?

> Cas9 can only be targeted to regions of the genome adjacent to a PAM sequence. DNA sequences that do not contain a PAM sequence would be inaccessible to Cas9-mediated gene editing.
> Because NGG occurs so commonly, it is relatively easy to find a PAM sequence in any region of the DNA that a scientist is interested in.

C. Compare the repaired DNA sequence from your paper model to wild-type sequence. On the "your result" sequence below (indicated by the arrow), cross out the DNA bases that were deleted when you repaired the DNA.

This answer depends on what the students chose to do in Step 7 where they model NHEJ. There are 36 possible sequences given the parameters that students can chose to delete between 0-5 bases on either side of the cut site.

D. Is your resulting sequence the same as any of the mutant sequences that scientists found after using this guide RNA?

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F. NHEJ introduces random mutations. Considering this, why do you think it is important for scientists to sequence the cell's DNA after CRISPR/Cas genome editing?

Scientists need to sequence the cell's DNA after CRISPR/Cas genome editing to determine the specific mutations introduced after NHEJ. It is also possible that Cas9 did not cut the target DNA or for the DNA to be repaired without the introduction of mutations.

G. Compare your guide RNA sequence with your classmates'. How many different guide RNAs did your class design to disrupt just this short region of the *BCL11A* regulatory DNA?

This answer depends on which PAM site the students chose to use in Step 9 where they design a guide RNA. Six of the PAM sites lead to Cas9 cutting within the 'Achilles heel' zone of the *BCLA11* regulatory DNA.

H. Why is it helpful for scientists that the Cas9 PAM sequence is relatively common in the genome?

Because the guide RNA must be adjacent to a PAM, having a common PAM sequence means that there are typically numerous potential guide RNA sequences that can be used to disrupt even a short target DNA sequence.

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