

CUT! How Does CRISPR Work?

Brainstorming worksheet — *Answer key*

1. CRISPR-Cas9 Mechanism

The first step of this brainstorming session is to dissect the molecular mechanism of CRISPR-Cas9, so you are able to visualize each individual step and the actions of each involved component in your stop-motion animation.

You can view these resources if you need a reminder of how CRISPR-Cas9 works:

- This video demonstrates how gene editing with the CRISPR-Cas9 technology works on a molecular level: <https://www.youtube.com/watch?v=2pp17E4E-O8>
- This simulation shows how CRISPR-Cas9 initiates the DNA double-strand break in the target region: https://www.labxchange.org/library/items/lb:LabXchange:b1057265:lx_simulation:1
- This simulation shows the two alternative pathways for how the created double-strand break in the DNA is repaired: https://www.labxchange.org/library/pathway/lx-pathway:d8553216-ea78-48aa-91e7-bbf007966b50/items/lx-pb:d8553216-ea78-48aa-91e7-bbf007966b50:lx_simulation:f1d73541

Discuss and answer the following questions about CRISPR-Cas9 within your group.

- ? What are the main components involved in the CRISPR-Cas9 editing mechanism?
- ? What is the function of each component?

Component	Function
<i>Single-guide RNA</i>	<i>Carries a specific nucleotide sequence that guides the Cas9 nuclease to the matching sequence in the target DNA.</i>
<i>Cas9</i>	<i>The nuclease protein induces a double-strand break of the DNA at the target site.</i>
<i>sgRNA-Cas9 complex</i>	<i>The sgRNA and the Cas9 can identify and cut the target DNA only when bound together in a complex.</i>
<i>PAM sequence</i>	<i>This short nucleotide sequence signals the single-guide RNA where to bind on the target DNA.</i>
<i>Target DNA</i>	<i>Part of the double-stranded DNA that includes the target sequence that will be edited.</i>
<i>DNA repair template</i>	<i>This DNA sequence is inserted into the cut target DNA to replace or correct a certain DNA sequence.</i>

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? How does the sgRNA-Cas9 complex find its target?

The Cas9 nuclease combines with the single-guide RNA to form an sgRNA-Cas9 complex. The sgRNA is key to finding the right target sequence within the target DNA. It carries the specific nucleotide sequence that guides the Cas9 nuclease to the matching sequence in the target DNA. The sgRNA-Cas9 complex scans the target DNA for a PAM sequence to lock on to. The CRISPR-Cas9 complex checks for precise base pairing between the sgRNA and the target DNA. Once the sgRNA matches with the target DNA, the Cas9 nucleases are activated and the DNA is cut. Precise base pairing between the sgRNA and the target DNA allows for a very high degree of target specificity.

? How does the sgRNA-Cas9 complex cut the target DNA?

When the correct target sequence has been identified and the sgRNA-Cas9 complex has bound to the target DNA, the two nuclease domains of the Cas9 protein are activated, which will each cut one strand of the double-stranded DNA.

? What happens after the DNA is cut?

Once the DNA is cut, the cell employs one of two repair mechanisms to fix the double-strand break. The first repair mechanism is more error-prone and usually leads to insertion or deletion of nucleotides at the target site. This results in a disruption and inactivation of the entire target gene. The second repair pathway usually involves a repair template that allows the introduction of specific nucleotide sequences or whole genes into the target site. This pathway results in gene modifications rather than gene disruptions.

? What is the sequence of events that is happening up until the DNA is repaired again?

[See table on next page.]

? What do you think are the most important parts to include for each step?

[Answers will vary.]

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Answer Key

? What is the sequence of events that is happening up until the DNA is repaired again?

Step	Description
1	Once the Cas9 nuclease, the sgRNA, the DNA target, and a potential DNA repair template come together in the host cell, the sgRNA combines with the Cas9 nuclease to form an sgRNA-Cas9 complex.
2	The sgRNA-Cas9 complex scans the target DNA for a PAM sequence (5'-NGG-3') to lock on to.
3	Once a PAM sequence is found, the sgRNA-Cas9 complex unwinds the DNA double helix of the target DNA.
4	The CRISPR-Cas9 complex checks for precise base pairing between the sgRNA and the target DNA.
5	Once the sgRNA matches with the target DNA, the Cas9 nucleases are activated.
6	The Cas9 nucleases create a double-stranded break in the target DNA 3 base pairs upstream of the PAM sequence, which results in two separate DNA fragments.
7a	The double-strand break is repaired by the homologous direct repair (HDR) pathway in the presence of a DNA repair template. This results in the insertion of the DNA repair template in-between the cut DNA fragments.
7b	The double-strand break is repaired by the non-homologous end joining (NHEJ) pathway in the absence of a DNA repair template. This can result in an insertion or deletion mutation (indels) at the cut site after joining both DNA fragments, thus inactivating the targeted gene.

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Answer Key

2. Stop-Motion Animation

The second step of this brainstorming session is to create a plan and make some basic decisions about the stop-motion animation you will create.

Discuss and answer the following questions within your group.

? *What materials could you use to model each component of the CRISPR-Cas9 mechanism?*

[Answers will vary.]

? *How will you explain what is happening in the video? Will you use onscreen text and/or a narration for your animation?*

[Answers will vary.]

? *How will you break down tasks so that each group member does an equitable amount of work?*

[Answers will vary.]