

How the Cas9 Protein Functions

Introduction

Once you understand the CRISPR-based adaptive immunity system that has evolved in bacteria to protect them from viruses, you are ready to look more closely at the **Cas9 protein** and understand how it functions in this system.

In this activity, you will explore the role of Cas9 by constructing a schematic model of the enzyme as it first binds to and then cuts a specific sequence of double-stranded viral DNA.

But before we jump into the modeling, here are a few things to think about:

- 1. CRISPR is a big deal.** It has already revolutionized the way biology is being done in laboratories all around the world. And yes, we will begin to edit the human genome with this technology in your lifetime.
- 2. There are legitimate ethical questions** that should be considered as we begin to think about editing the human genome, and as a future voter, you will be asked to weigh in on these issues.
- 3. So remember to thank your teacher** for their recognition of the importance of introducing you to this topic. With a sound understanding of how the technology works, you will be well-prepared to participate in these ethical discussions in the future, and to explain to others how this system works.

Before you begin constructing the schematic Cas9 model, review your understanding of the following CRISPR vocabulary by writing a brief description of each term.

CRISPR locus: _____

CRISPR array: _____

Spacer sequence: _____

How the Cas9 Protein Functions

CRISPR repeats: _____

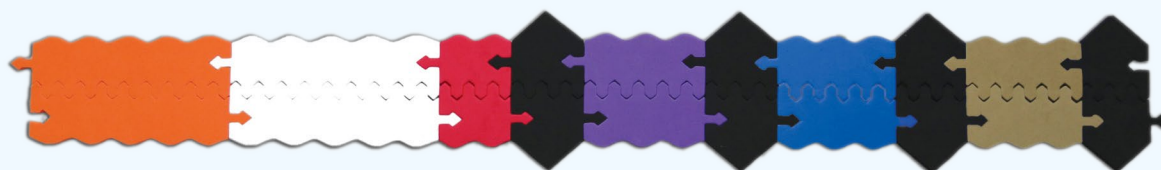
tracrRNA: (pronounced "tracerRNA"): _____

Guide RNA: _____

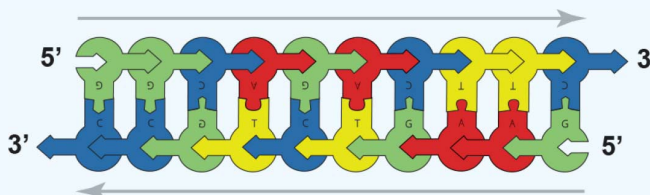
What does the CRISPR acronym stand for?

C _____ R _____ I _____ S _____ P _____ R _____

Here is a schematic illustration of the CRISPR locus. Label the components of this illustration with the words represented by the CRISPR acronym.



One more thing before we begin -- you will be using foam nucleotides to represent both RNA and DNA sequences in this model. Polarity is important. Note the 5' and 3' ends of the sequence represented below. And remember, whenever two strands of DNA, or RNA, form a double-stranded structure, the two strands must be anti-parallel.



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Modeling the CRISPR Cas9 Protein

STEP 1: Construct your viral DNA sequence.

Using the multi-colored foam deoxyribonucleotides (DNA), construct a model of a double-stranded viral DNA with the following nucleotide sequence:



STEP 2: Construct your crRNA (CRISPR RNA).

crRNA is composed of a spacer sequence and a portion of the repeat RNA. Using the purple-colored ribonucleotides (RNA), construct a model of the first 20 nucleotides of the crRNA with the following spacer sequence (note that this is complementary to a segment of the viral DNA):



Then extend the 3' end of your spacer RNA sequence by adding the black RNA segment. This black sequence represents a segment of the CRISPR repeat sequence. This black repeat sequence, along with the purple spacer sequence represents the crRNA. crRNA is transcribed from the CRISPR array, and contains viral sequences at its 5' end, and repeat sequences at its 3' end.



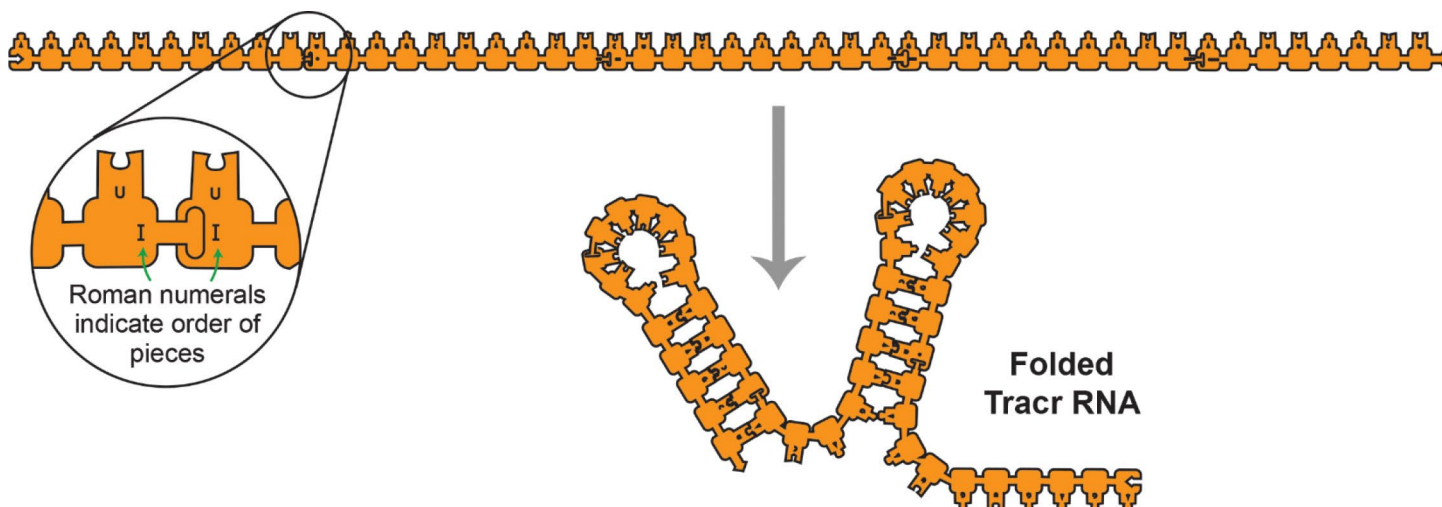
STEP 3: Construct a model of tracrRNA.

tracrRNA is transcribed from the tracr gene. But it is **NOT** a messenger RNA. It is **NOT** translated into protein. Instead, it functions as a structural RNA. It folds up into a complex 3D shape, much like a protein. The tracrRNA is then bound in a very specific way by the Cas9 protein. (You will see in the next step that the 5' end of the tracrRNA forms complementary base pairs with the 3' end of the crRNA.)

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How the Cas9 Protein Functions

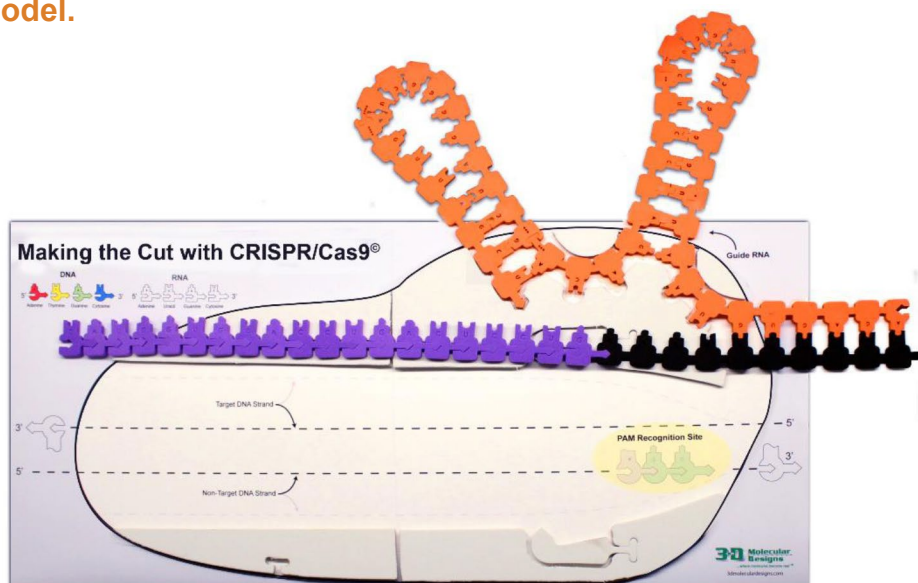
Join each of the five orange-colored RNA fragments together in the correct order, by matching the numbers at the end of each segment. Once you have made one long continuous sequence of tracrRNA, examine the sequence (starting at the 3' end) to find the complementary base pairs that results in the two "hairpins" shown below. This is simply a two-dimensional model of the very complicated 3D shape of the actual tracrRNA.



STEP 4: Construct the dual guide RNA and add it to the Cas9 protein model.

Dual guide RNA is made up of two RNAs – the tracrRNA and the crRNA. The 5' end of the tracrRNA is complementary to the 3' end of the crRNA. Add this dual guide RNA to the Cas9 protein as shown in the photo to the right. (Start by positioning the 3' end of the tracrRNA into the slot in the Cas9 model.)

Once you have added the guide RNA to Cas9, the purple viral RNA sequence is now ready to interrogate the target strand of double-stranded viral DNA. If the guide RNA spacer matches the viral DNA, Cas9 will then cut both strands of viral DNA.



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How the Cas9 Protein Functions

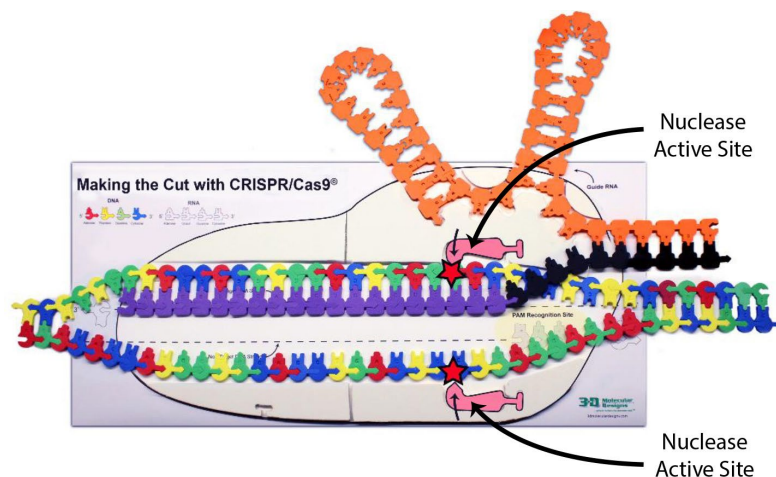
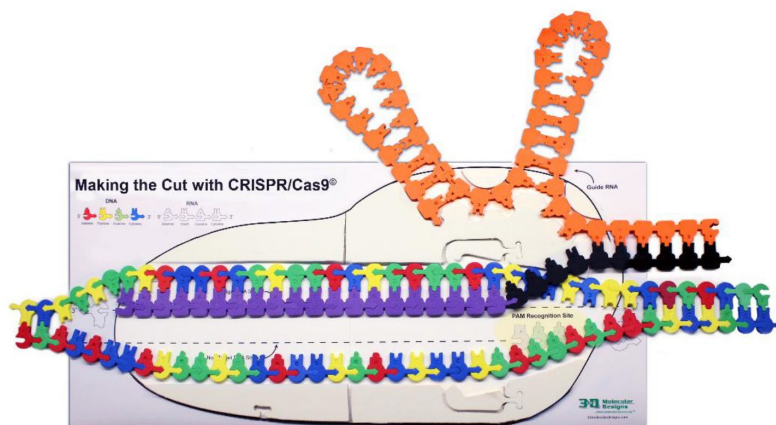
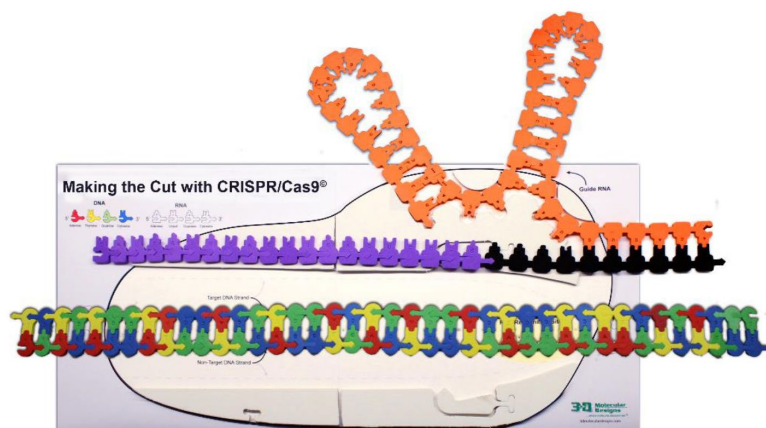
STEP 5: Add the double-stranded viral DNA sequence to the Cas9 protein.

The Cas9/guide RNA complex binds double-stranded DNA, but only at a PAM site. A PAM site (Protospacer Adjacent Motif) is the sequence 5'-NGG-3', where N is any nucleotide. Therefore, take the double-stranded DNA sequence that you assembled and add it to the schematic Cas9 model with a PAM site positioned over the site indicated on the Cas9 model. (Note that there are several other PAM sequences found in this double-stranded DNA sequence).

Once the DNA is bound by Cas9 at the indicated PAM site, separate the two DNA strands, so that the purple viral RNA sequence can interrogate the top/target DNA strand.

If the viral RNA sequence is complementary to the top/target DNA strand, the two separate nuclease active sites of Cas9 are activated, and both strands of DNA are cut. The cut occurs 3 bases upstream of the PAM site.

As a result, Cas9 has created a double-stranded, blunt end cut at a specific sequence in the viral DNA. **Congratulations**, you have just destroyed the viral genome, and prevented the infection of the bacteria.

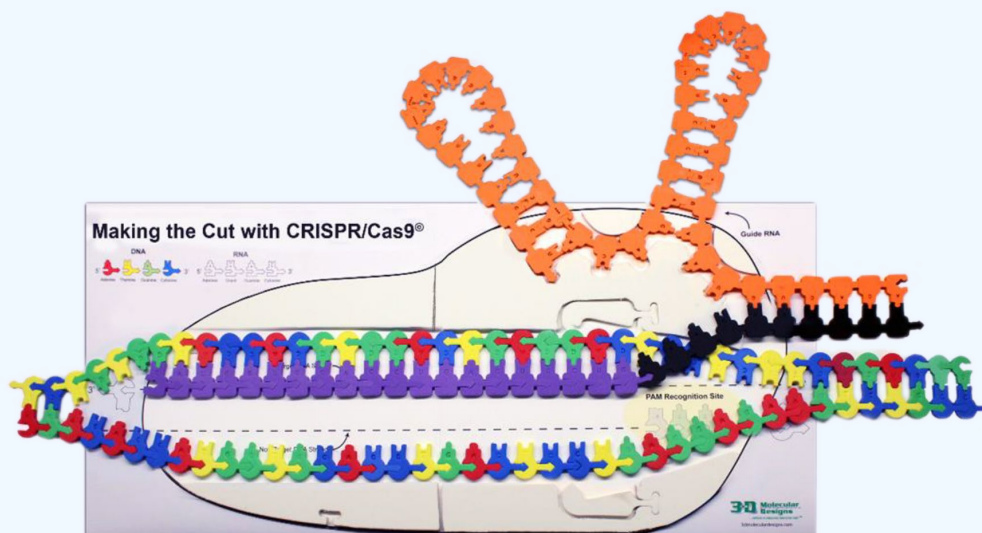


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How the Cas9 Protein Functions

A Final Review

Let's review this one last time. Demonstrate your understanding of how the Cas9 endonuclease works by labeling the photo of the schematic model shown below, using the CRISPR vocabulary from this exercise.



Are you wondering how this schematic model of Cas9 relates the actual 3D structure of the Cas9 protein complex? Below is a photo of a physical model of this protein. Note that it is the same model being held in the photo of Jennifer Doudna on the left. You may know that Dr. Doudna shared the 2020 Nobel Prize in Chemistry with her colleague Dr. Emmanuelle Charpentier for their work in figuring out how this protein works.



Cas9 is a large protein composed of 1053 amino acids that fold up into many functional domains. Endonucleases are potentially dangerous to a cell and therefore are only activated if the guideRNA matches perfectly with the viral DNA sequence.

