Engineering the Cas9 Protein

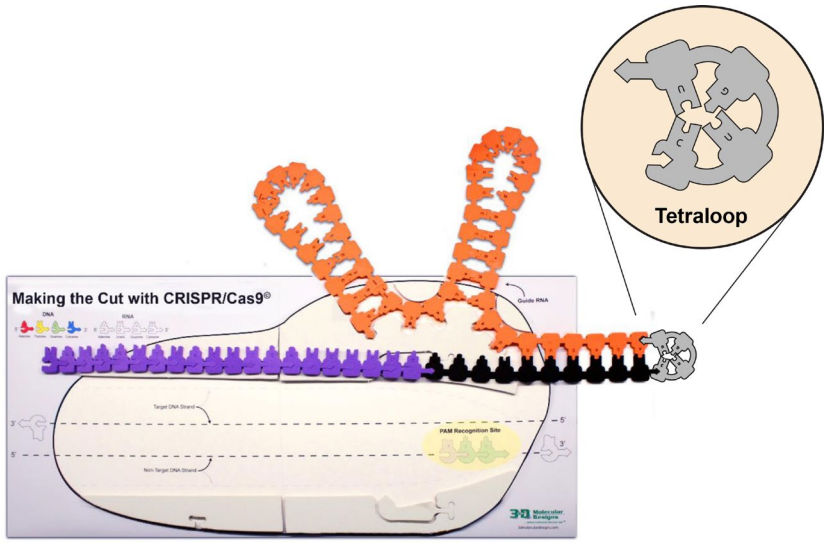
Introduction

The Cas9 protein was descovered as part of the CRISPR adaptive immunity system found in some bacteria, in which it identifies and cuts invading viral DNA. But scientists are most excited by how the Cas9 protein might be adapted for use in research labs. A natural biological system or process that is adapted for use as an engineering tool is referred to as ***Biotechnology***.

Based on your knowledge of CRISPR-Cas9, how might it be used as a biotechnology tool in research labs and ultimately as a gene editing tool?

Cas9 Biotechnology

There are several ways Cas9 is already being used as a biotechnology tool.



1.) Knocking Out a Gene

Rather than cutting viral DNA, scientists can load the Cas9 protein with an engineered guide RNA that matches a DNA gene from a non-viral organism. The Cas9 protein will then find the matching DNA sequence and make a double stranded cut that will inactivate – or ***Knock Out*** - the gene.

The 2020 Nobel Prize in Chemistry was awarded to ***Jennifer Doudna*** and E***mmanuelle Charpentier*** for their work in this field. They designed a linker called the ***Tetraloop***, that connects the two segments of the guide RNA (the tracrRNA and crRNA) into one single strand. You can add this engineered feature to your model using the gray tetraloop piece.

How might the ability to knock out a gene be used as a biotechnology tool?

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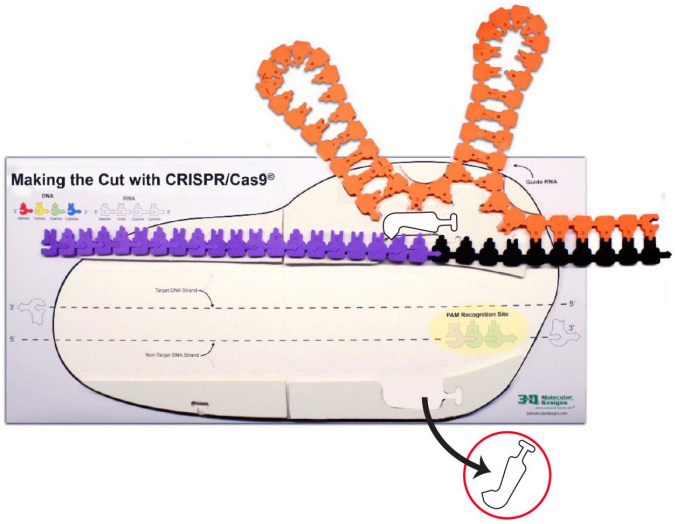
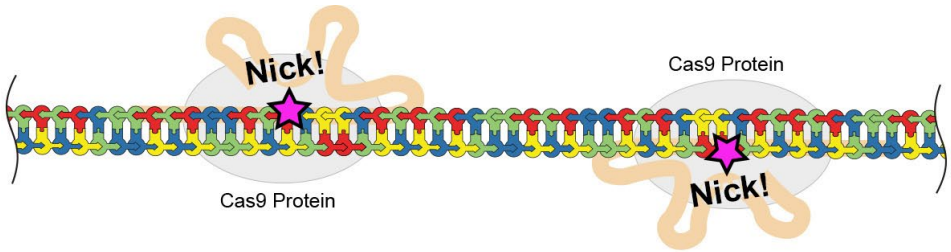
Why might the tetraloop make Cas9 a better biotechnology tool?

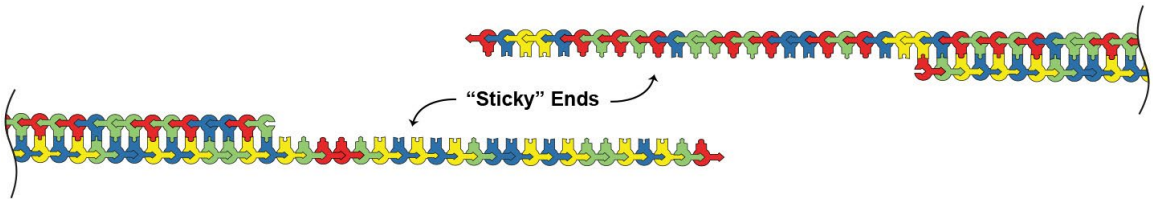
2.) Nicking One Strand



Scientists can inactivate one of the two ***nuclease domains*** in the Cas9 protein, resulting in a single stranded "***nick***" rather than a double-stranded cut. You can represent this engineered feature on your model by removing one of the two nuclease domains.

Using two Cas9 proteins, each with one inactivated nuclease domains and a different guide RNA that will target a slightly different spot in a segment of DNA, scientists can even produce cut DNA with ***Sticky Ends***, as shown below.





How might the ability to make "sticky end" cuts in DNA be used as a biotechnology tool?

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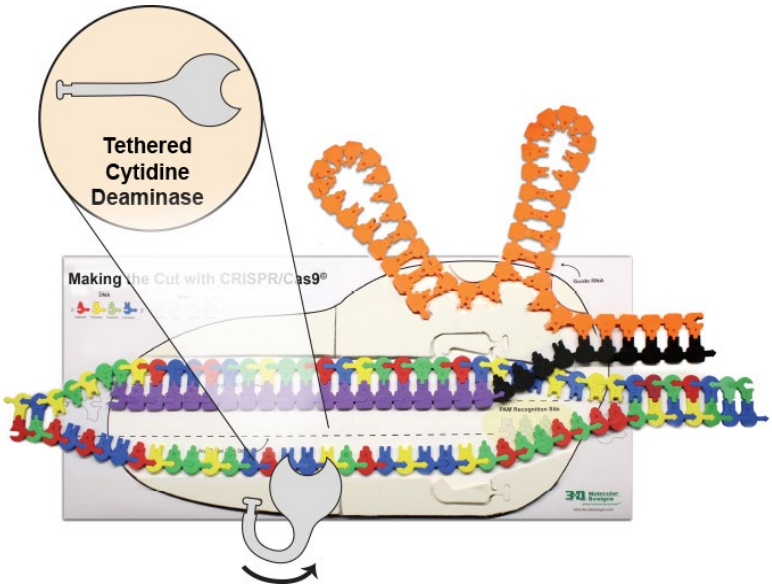
3.) Delivering Fusion Proteins to a Specific Location in a Genome

Other proteins can be fused to Cas9 without impairing the function of Cas9 or the fused protein. In this application both nuclease sites of Cas9 are inactivated. This “dead Cas9” will then use its single guide RNA to target a specific gene, but not cut it. It will simply deliver the fused protein to this specific gene. If the fused protein is a transcription factor, this approach can be used to regulate the expression of this gene. If the fused protein is a DNA methylase, this approach can be used to alter the epigenetic state of a specific gene.

How could you fuse (tether) another protein to Cas9?

4.) Base Pair Editing

Scientists can tether a specific type of protein, called a ***Base Editor***, to the Cas9 protein. Base editors are able to convert one type of nucleotide (A, T, G or C), into another. For example, the base editor ***Cytidine Deaminase*** can convert a cytosine (C) base to a thymine (T) base. You can represent this engineered feature on your model by adding the tethered cytidine deaminase piece, which can then modify a nearby C to a T.



How might being able to make precise single nucleotide changes to a specific section of DNA be used as a biotechnology tool?