CRISPR Adaptive Immunity Kit©

3-D Molecular Designs ...where molecules become real ™

Teacher Notes

What You Can Teach with the CRISPR Adaptive Immunity Kit©

The **CRISPR Adaptive Immunity Kit©** was developed to explain how CRISPR functions as an adaptive immunity system in bacteria, protecting them from virus infection.

Specific questions/concepts/topics that can be addressed with this kit include:

- What is a virus?, and more specifically, what is a bacterial virus, i.e., a bacteriophage?
- CRISPR systems are one of several defenses that bacteria have evolved to protect themselves from viruses.
- The prototypical CRISPR system that everyone talks about is the Cas9 system found in Streptococcus pyogenes. But many variants of this system have evolved in other bacteria – each with Cas9-like components with a rich variety of functions and mechanisms. As we often say . . . Life is Complicated.
- The CRISPR system in a bacteria includes a mechanism to. . .

  (I) Detect the presence of a double-stranded DNA genome of a virus that is attempting to infect it.

  (II) Integrate a short fragment of this viral genome (~20 base pairs) into the CRISPR array in the bacterial genome.

  (II) When the CRISPR array is expressed, a single-stranded guide RNA is created that is complementary to a short segment of the viral genome.

  (IV) This guide RNA programs an endonuclease, Cas9, to seek out the infecting viral genome and cut it, thereby preventing infection.
- The CRISPR-Cas9 endonuclease (and its related endonucleases from other bacteria) has been further engineered to become a powerful new tool that molecular biologists are using to edit many plant and animal genomes, including the human genome (see below).

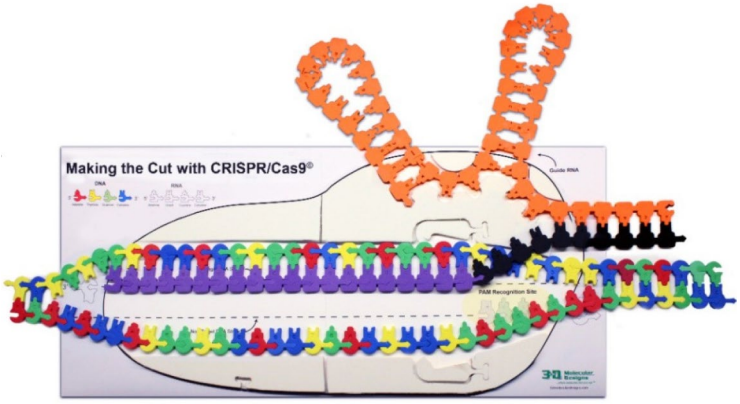
Connections to other related topics include:

- Restriction enzymes represent another virus defense system in bacteria.
- Compare and contrast this CRISPR-based immune system in bacteria with our own adaptive immune system, based on antibodies.
- Biotechnology: how is the CRISPR-Cas9 endonuclease being engineered to edit the human genome?
- What is the structure of the CRISPR Cas9-endonuclease, and how does it work?
- CRISPR-Cas9 can seek out a unique 20 nucleotide sequence in the human genome and cut it. What happens next in order to edit the gene that has been cut?
- How has Cas9 been further engineered to become a more efficient genome editor?
- Just as the discovery of restriction enzymes in the 1970's revolutionized biology and led to the recombinant DNA era, CRISPR technology is currently revolutionizing how biology is being done today, at the level of eukaryotic genomes.

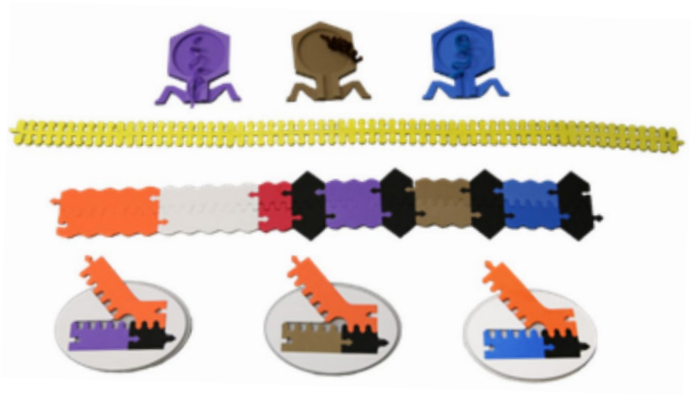
Student Handout

Teacher Notes - About the Making the Cut with CRISPR-Cas9 Kit©

CRISPR-Cas9 is an RNA-guided endonuclease that plays a central role in the adaptive immunity system that has evolved to protect bacteria from viruses.



To the right is 3DMD's **Making the Cut with CRISPRCas9©** kit, which was developed to provide your students with a way to think more deeply about how this enzyme works. Once your students understand how the protein binds to a specific sequence of double-stranded DNA and cuts it they will then be ready to think about how it can be used to edit the human genome.



BUT, we highly recommend that you use the **CRISPR Adaptive Immunity Kit©** to introduce your students to the CRISPR system **BEFORE** you use the schematic CRISPRCas9 model shown above to explore how the enzyme works. After your students have been introduced to the CRISPR system, they will be familiar with the many terms that are used to identify the various components on the Cas9 endonuclease (tracrRNA/guide RNA/spacer/repeat). This familiarity with CRISPR terms will allow them to focus on the mechanistic details of how the enzyme works.

Another major question faced by teachers who want to introduce their students to CRISPR is – **how do I connect this topic to what I teach?**

Although there are several answers to this question, **we suggest that you compare and contrast Cas9 with a restriction enzyme.** They are both endonucleases that can recognize a specific sequence of DNA and cut it, but they are different in two important ways:

1. Cas9 uses a guide RNA to recognize a specific sequence of DNA.
2. Text BoxCas9 can recognize a much longer sequence of DNA. This property allows Cas9 to recognize a statistically unique site in the 3.2 billion base pair human genome – which makes it useful editing large genomes.

A video discussing the comparison of Cas9 with a restriction enzyme is available at ***https://www.youtube.com/watch?v=VU346i-mhAo*** (from 7:46 -–19:30).

Student Handout

Teacher Notes - How to Generate Interest in CRISPR

Below is a suggestion as to how you might get your kids interested in this topic. We suggest that you challenge your class to consider the question: ***Could you have discovered CRISPR?***

The first report of what was later to become known as the CRISPR system appeared in a paper from a Japanese research group) in 1987. These researchers noticed an unusual pattern in the sequence of nucleotides that occurred just downstream from an E. coli gene they were studying. They had no idea what this sequence meant, only that it was interesting, and worth reporting to the scientific community.

Over the next ten years, other nucleotide sequences with this pattern were reported in the genomes of other bacteria. Eventually, this pattern was named CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats.

So, here is the challenge: ***Give your students the following sequence of nucleotides. . .***

GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAG
TGCGCCCGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCT
CACCGTCGTTGCCGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTT
GCAAACCTGGCTACCGGGCGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCA
TCATTCCACCTATGTCTGAACTCCCGGTTTATCCCCGCTGGCGCGGGGAACTCG

. . .and ask them if they can see the unusual pattern in this sequence of nucleotides.

If they can, they can be assured that they are smart enough to have discovered CRISPR, if only they had been lucky enough to be part of that Japanese research group back in 1987 (Y Ishino, H Shinagawa, K Makino, M Amemura, and A Nakata J Bacteriol. (1987) 169(12): 5429–5433).

You probably want to give this to your students just before a weekend and encourage them to work together to solve this puzzle. If all goes well, you will be ready to start the CRISPR lessons on Monday.

You will notice that this activity doesn't really explain how CRISPR functions as an adaptive immunity system in bacteria. But what I love about this activity is that if students are successful, they will have discovered a pattern of clustered regularly interspaced short palindromic repeats, i.e., **CRISPR**. With this information in their heads, they are ready to explore the CRISPR Adaptive Immunity Kit©.

A video describing this activity is available at ***https://www.youtube.com/watch?v=buEYPJf6aIM*** (from 9:15--23:35).