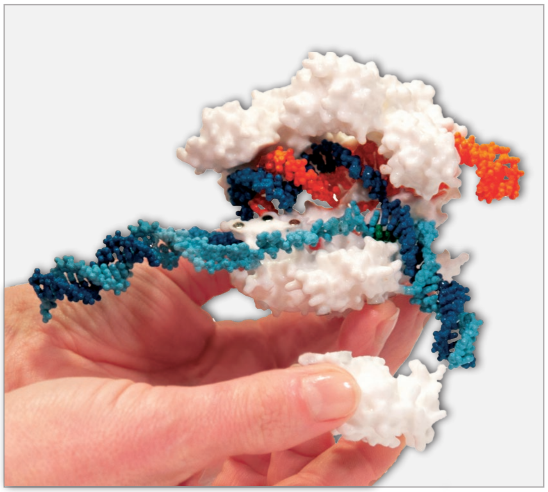
CRISPR Adaptive Immunity Kit©

Student Handout



Introduction

The history of **CRISPR** is a long road spanning nearly 30 years – with a few twists! Have you heard or read about CRISPR in the news? Do you know what the letters in the acronym mean? You'll not only discover what the CRISPR acronym represents, but how scientist discovered it and cutting-edge ways scientists are using it today.

Mind Dump

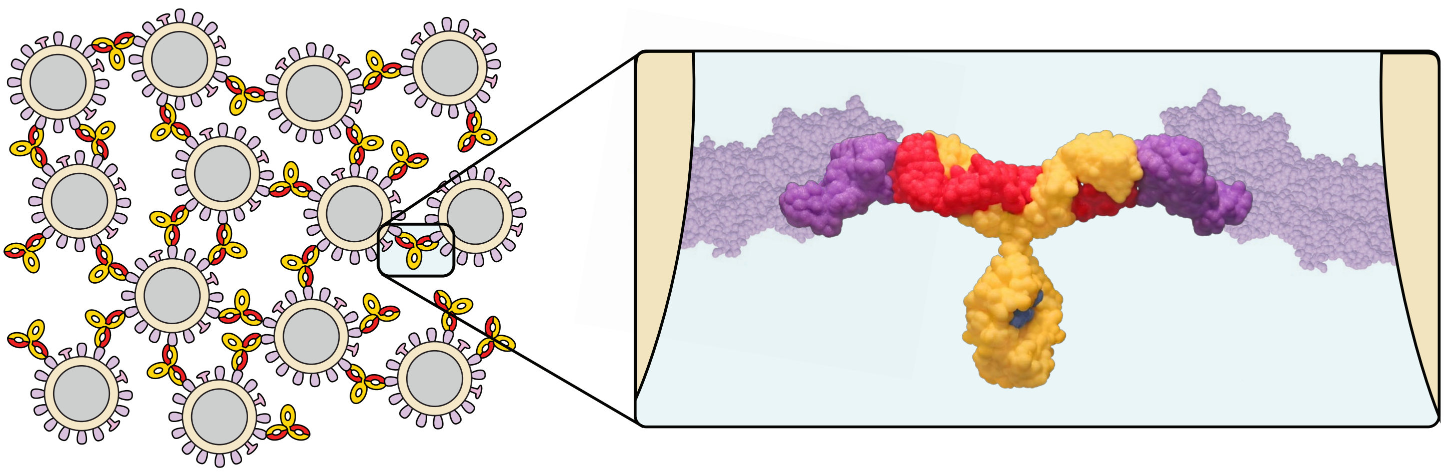
List everything you know CRISPR in the box below. What does the acronym stand for? What do the words mean? Who discovered CRISPR? When was it discovered? List whatever you know about CRISPR.

What questions do you have about CRISPR? List what you have ever wondered about CRISPR.

Adaptive Immunity System in Bacteria

Before you begin modeling how the CRISPR system functions as an adaptive immunity system in bacteria, let's examine **our own adaptive immune system** so that we can compare it to how a bacteria's adaptive immune system works.

Our human adaptive immune system is based on Y-shaped antibody proteins composed of two heavy chains and two light chains. The tips of the heavy and light chains are key to staying well, since tips bind to an area on a virus protein, called antigen binding site. When scientists make a vaccine it is designed to trigger our bodies to make specific antibodies that will bind to the antigen binding site - whether it is for influenza virus or another virus. The more specific the antibody tip is to the antigen, the better the antibody will neutralize that virus and keep us well.



When you get a viral infection, what do you think the virus trying to trick your cells into doing? Why does a virus need a host? Write your thoughts below:

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You have to get a flu vaccine every year to stay well. What changes about the flu virus each year that requires us to get a new vaccine? Why might the previous vaccine not work anymore?

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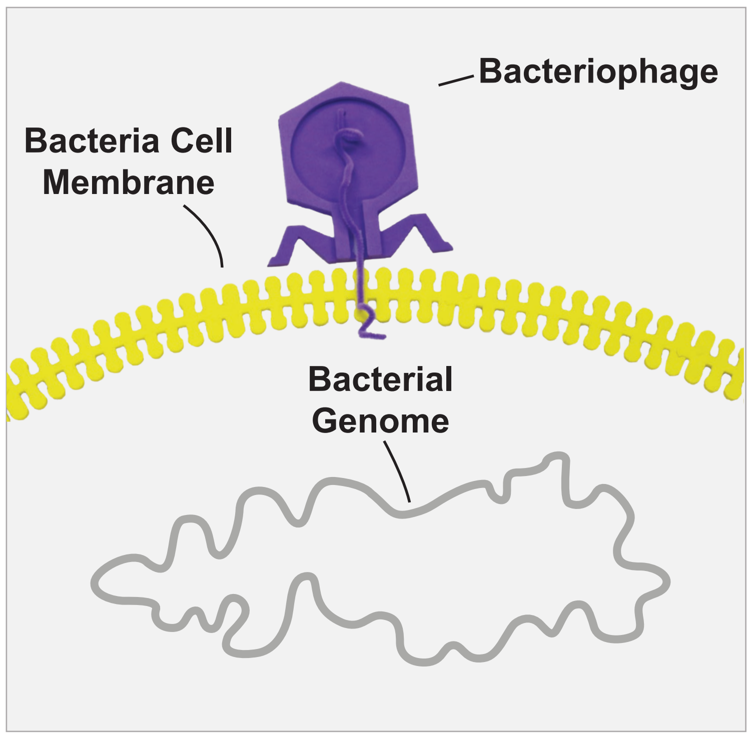
In what ways is our immune system “adaptive”? Write your ideas below:

How does the bacterial adaptive immunity system work?

Bacteria have been under constant attack by viruses for millions of years. The viruses are, called bacteriophages, or simply phages.

When a bacteriophage attacks a bacterial cell, like *E. coli,* the bacteria have just a short time to spring into attack mode to fend off the double-stranded DNA (dsDNA) the virus inserted through the bateria’s cell membrane. CRISPR is the adaptive immune system that evolved in some bacteria to survive repeated infections by phages.

If a bacteria survives the initial infection by a phage, it cuts out a short sequence of the virus’ genome (DNA) and inserts into a section of the bacteria’s own genome. This site is called the CRISPR locus. (See illustration on the next page. The image below the cell membrane represents double-stranded DNA.)



When that same phage attacks it in the future, the bacteria is able to transcribe a short section of the viral DNA within its CRISPR locus to deploy enzymes called endonucleases to seek and destroy the virus’ DNA.

If the bacteria hasn’t previously encountered this virus, the phage tricks the bacteria into making many copies of the phage. Once new bacteriophages are produced, the bacteria will burst open, releasing the thousands of new phages to attack nearby cells.

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Why is the system called CRISPR?

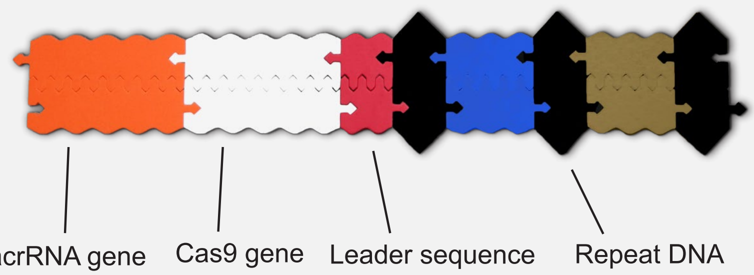
CRISPR stands for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats.

Palindromic repeats are short, repeating sections of DNA found in many bacteria.

This pattern of regularly spaced short palindromic repeats was first recognized by a group of Japanese researchers in 1987 and eventually led to our understanding of how this CRISPR system works.

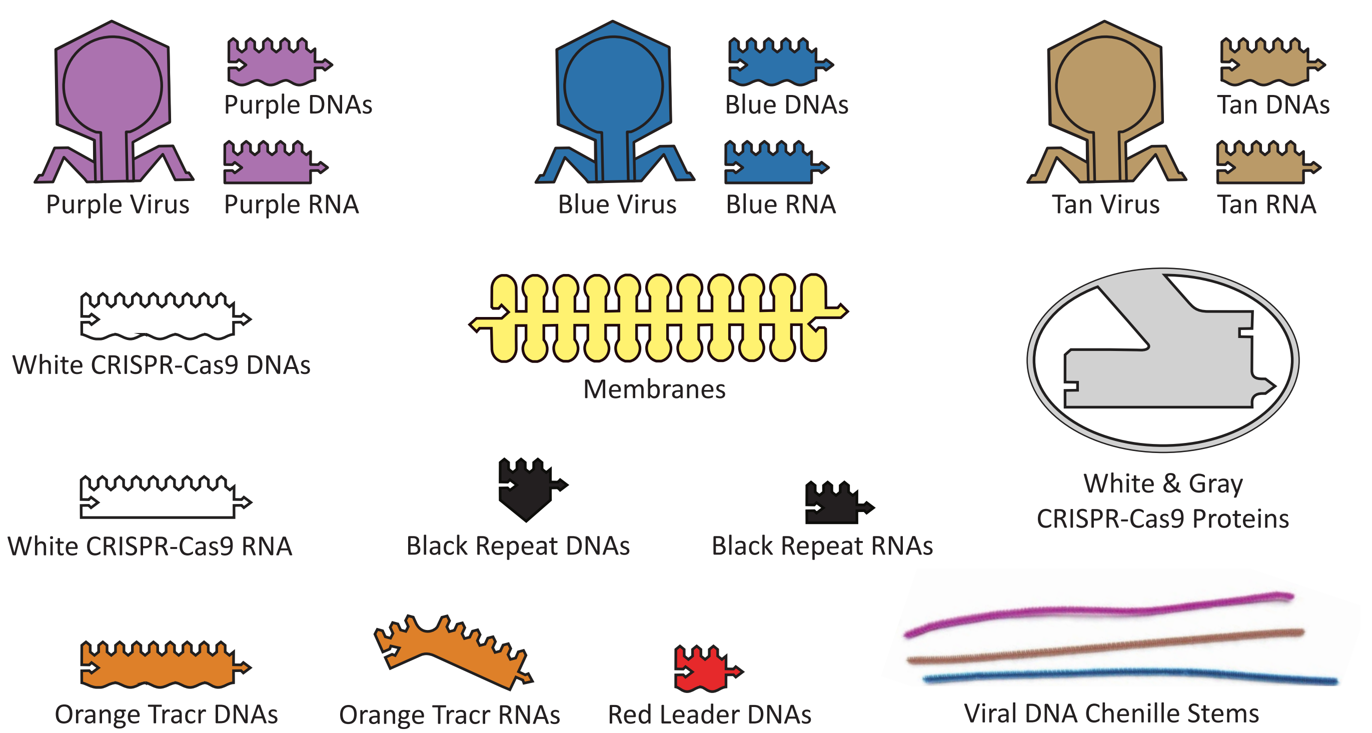
Circle structures in the illustration to the right that you think might be palindromic repeats.

CRISPR Locus

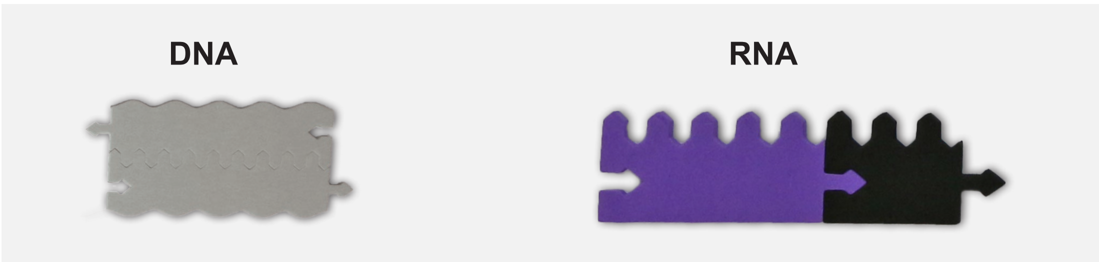


Review the kit pieces shown in the Contents and write your observations in the lines on the next page.

Contents List



What did you observe about the virus DNA and RNA nucleotides?



What other structures follow this pattern?

Scientists often make inferences from their observations.

What can you *infer* about the color coding of the virus DNA and RNA pieces?

What other purple, blue, and tan nucleotides do you see?

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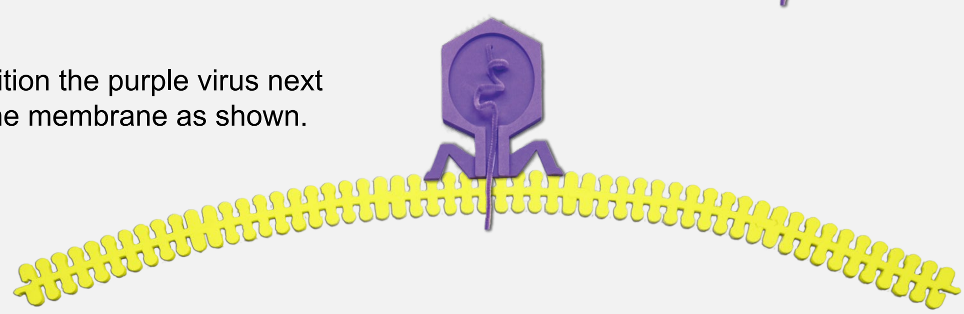
Setting the Scene

Assembling the Bacterial Membrane and its DNA

1. Begin assembling your bacterial cell by joining the five membrane sections together to show a portion of the cell membrane (see photo to the right).

2. Add the purple chenille stem to the purple virus. Squiggle about one fourth of the purple chenille stem inside the purple virus center cavity. Use the remaining one third to cross the membrane and go into the bacteria.

3. Position the purple virus next to the membrane as shown.



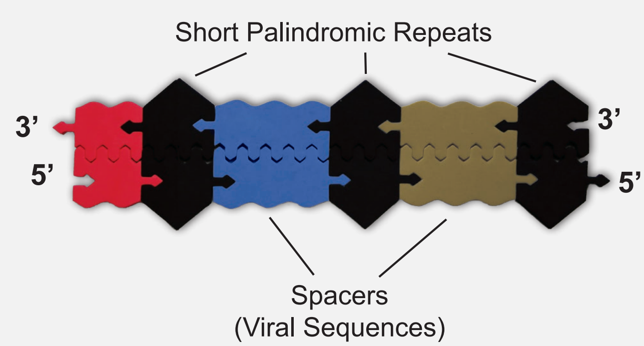
Adaption

Assembling the CRISPR Array

Join the single-stranded DNA sequences together with their matching pieces to form double-stranded DNA. The top arrow in the pieces should point to your left and the bottom arrows in the pieces should point to your right. On the next page, you will join your **CRISPR Array** with other genes to create the **CRISPR Locus**.

The black diamond-shaped DNA sequences represent the ***short palindromic repeats***. The tan and yellow pieces represent the ***spacer*** sequences.

The CRISPR Array



Why do the arrows point in opposite directions?

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How many viruses' DNA are represented in your model of the CRISPR Array?

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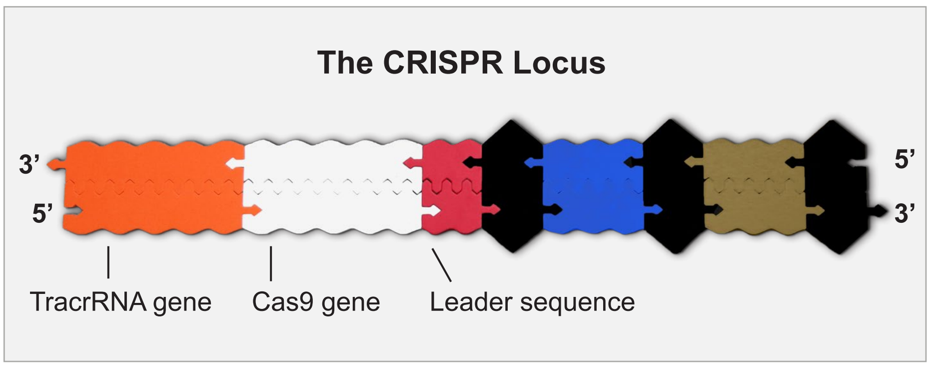
Assembling the CRISPR Locus

The CRISPR Array is found within a small section of the bacterial DNA known as the CRISPR Locus. In the CRISPR Locus, the CRISPR Array is preceded by a sequences known as the CRISPR-associated genes (Cas). These Cas genes include the TracrRNA gene (pronounced "tracer RNA"), white Cas9 gene, and the leader sequence. Add these Cas genes to your CRISPR Array to model the CRISPR Locus (shown right).



Aquisition

Modeling How Bacteria Acquire Viral DNA

The purple virus is attacking your bacteria. What does your bacteria need to do?

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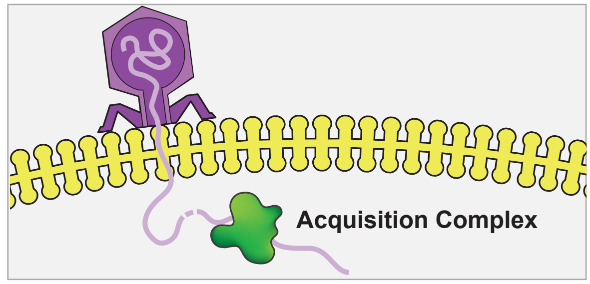
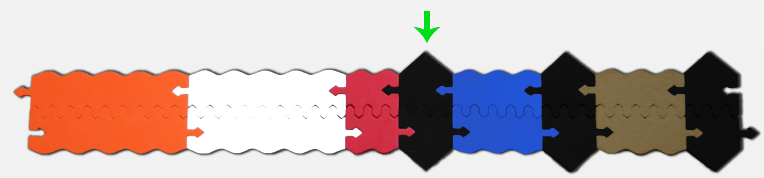
Bacteria send ***an acquisition complex*** (a multi-subunit complex of several proteins) to cut and remove a short segment of the double-stranded viral DNA before taking it to the CRISPR Locus.

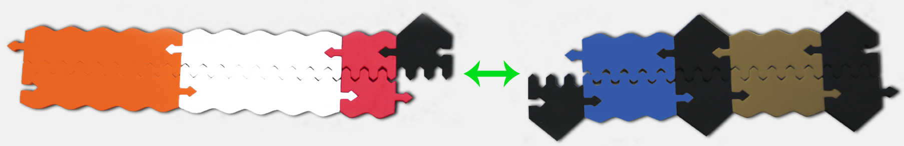
After the cut, the acquisition complex - represented in this activity by your hands - moves the purple spacer sequence to the CRISPR array.



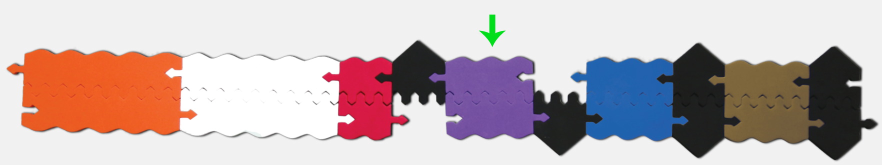
1. Locate the black repeat immediately to the right of the red leader sequence.



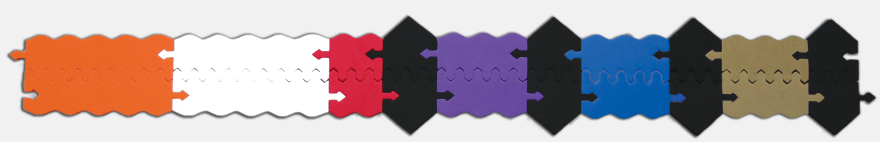
2. Separate the black repeat's DNA into two strands of single-stranded DNA and move apart the two sections.



3. Insert the purple spacer DNA between the two segments and join to the black repeat single strands.



4. Use the remaining black repeat DNA pieces fill in the gaps created by the acquisition of the purple DNA.



DNA polymerase is an enzyme that fills in the missing DNA nucleotides. What other process requires DNA polymerases to fill in DNA nucleotides?

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This adaptive immunity process of inserting viral DNA into the bacteria's own DNA genome results in the CRISPR locus being reset and ready for the next viral infection. The CRISPR array could be thought of as the bacteria's ***immunization record.***

Based on the process you used to insert the purple viral DNA which color represents the oldest infection?

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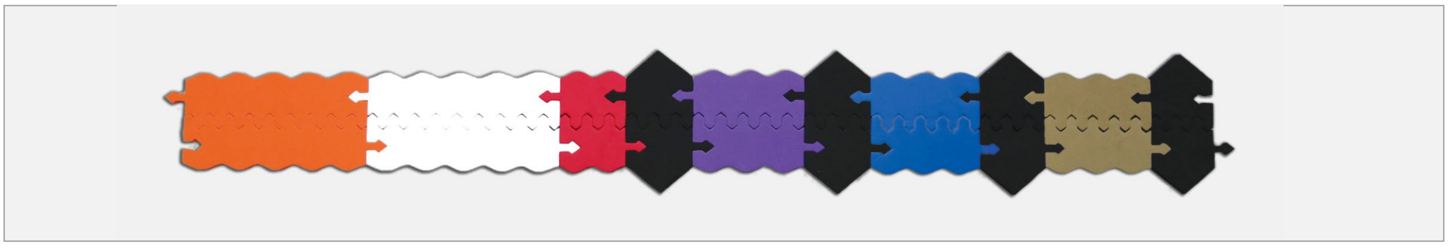

Expression

Assembling the Cas9 Endonuclease

Once a bacterium acquires a fragment of the purple viral sequence into its CRISPR array, it is ready to express these genes to assemble an ***RNA-programmed Cas9 endonuclease*** that will protect the virus from future attacks by the purple virus. An endonuclease is a type of enzyme.

What do you know about enzymes?

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Using your prior knowledge, what processes do you think organisms use to *express* genes or DNA?

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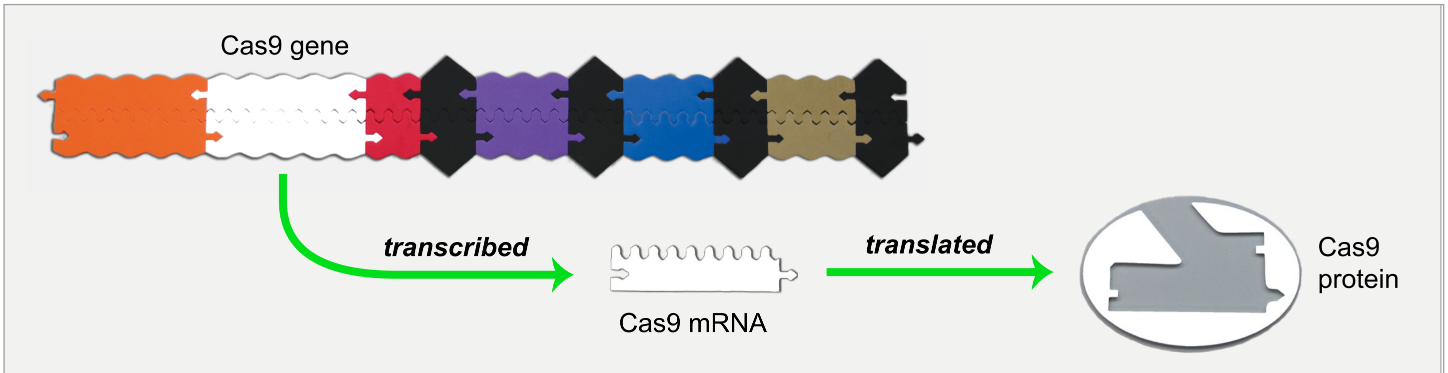
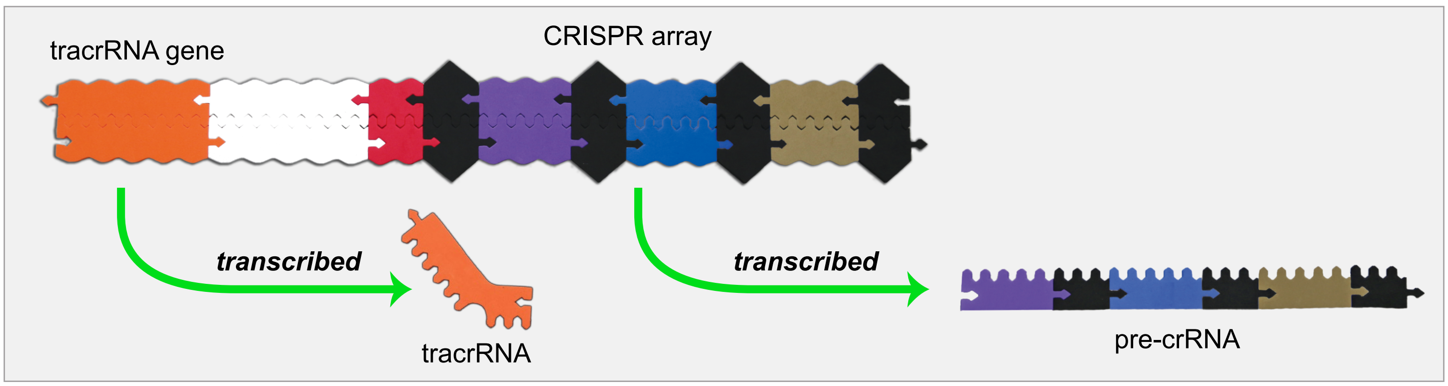
It is best to think of the expression of the CRISPR Locus in two phases. ***First***, the ***Cas9 gene*** is ***transcribed*** into mRNA and then this mRNA is ***translated*** into the Cas9 protein.



Second, the other genes of the CRIPSR Locus are transcribed. . . but not into mRNA. Instead, they are transcribed into structural RNAs that are not used to translate protein. The orange tracrRNA gene is transcribed into the tracrRNA structural RNA and the CRISPR aray is transcribed into the pre-crRNA.



What parts of the CRISPR locus DNA will be transcribed into RNA?

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What parts of the CRISPR locus will be translated into protein?

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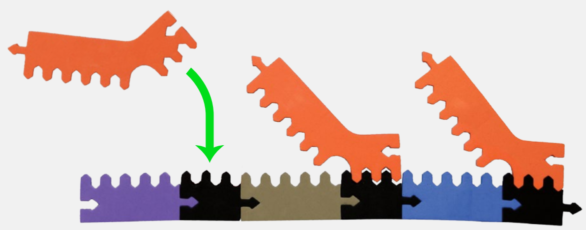
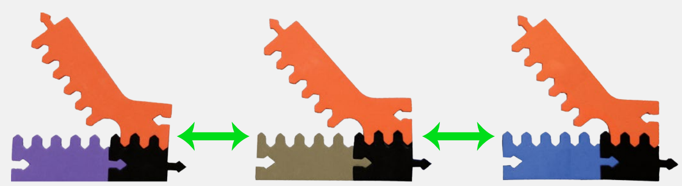
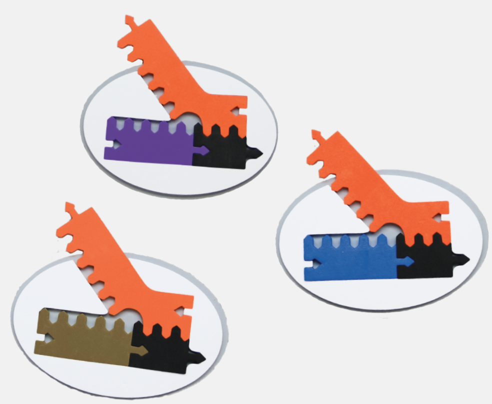
1 In the final steps, transcribed copies of the orange tracrRNA base pair with the black repeats in the pre-crRNA.



2 The crRNA/tracrRNA complex is then cleaved at the 3' end of each repeat sequence to produce three separate dual-RNA guides, each containing a spacer sequence representing a different virus.



3 These dual-RNA guides are then bound by a Cas9 protein to generate the RNA-programmed Cas9 endonucleases that are now ready to search out and destroy invading virus genomes.



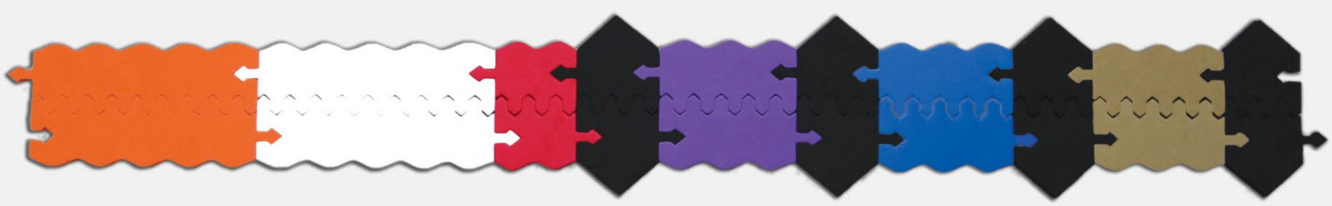
The fully assembled RNA-programmed Cas9 endonucleases are now ready to search out and destroy invading virus genomes. They do that by cutting the viral DNA at a specific location, using the small section of viral DNA held in the Cas9 protein as a guide. This final step in the CRISPR adaptive immunity mechanism is often referred to as ***Invader Silencing***.

A Final Review

Of the CRISPR Adaptive Immunity System

Examine the photo below and the bulleted word list. Label the illustration with the bulleted words.

- ***tracrRNA gene***
- ***Cas9 gene***
- ***Spacer***
- ***CRISPR locus***
- ***Repeat***
- ***Leader***



What did you think of the CRISPR Adaptive Immunity System?

Thinking like a scientist, what do you think about the adaptive immunity process you just modeled? Are you skeptical about any of the process? Did you find anything unbelievable? Are you wondering if this is all true? How did it come to work this way? How did scientists figure it out?

Use the space below to write down your thoughts - and most important your questions you are wondering about.

Acquisition of New Spacers: Storyboard

Now that you have modeled the CRISPR adaptive immunity process, use the four boxes below to illustrate the steps of the acquisition of bacteriophage DNA as if it were a comic strip. On the lines beside each box, describe what is happening in each drawing. Use and underline the following words: **bacteriophage, docking, leader sequence, repeat sequence, spacer, insertion.**

Big idea of what happened in this step:

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More detailed description of what happened:

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Big idea of what happened in this step:

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More detailed description of what happened:

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Big idea of what happened in this step:

More detailed description of what happened:

Our bacteria has survived the initial attack by the purple phage, and a short segment of the phage genome (the purple spacer) is now integrated in to the CRISPR array of the bacteria. But what happens next to allow this bacteria to quickly respond to a subsequent infection by that phage and destroy it?

Think back to what we know about genetic information flows from DNA to RNA to protein. Briefly describe how this works.

So, with that basic flow of genetic information in mind, ***how do you think the bacteria might use its integrated purple spacer to protect itself from another attack by the purple phage?***

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Now that you have modeled how a bacteria will use the CRISPR locus to create a CRISPR/Cas9 complex that the bacteria will then use to fend off invading bacteriophages in the future, use the space below to write down any questions that you thought of while modeling the process.

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Building and Deploying the CRISPR Cas9 Complexes: Storyboard

Illustrate in the boxes below the steps of transcription/translation of the CRISPR locus as if they were a comic strip. On the lines beside each box, describe what is happening in each drawing. Use and underline the following words: **transcription, translation, ribosome, cas9 protein, RNA polymerase, CRISPR array, tracrRNA, spacer, repeat, CRISPR-RNA, guideRNA .**

Big idea of what happened in this step:

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More detailed description of what happened:

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Student Handout

Big idea of what happened in this step:

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More detailed description of what happened:

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Big idea of what happened in this step:

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More detailed description of what happened:

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Big idea of what happened in this step:

More detailed description of what happened:

Congratulations, you now understand how the CRISPR-based adaptive immunity system works in bacteria! Now compare and contrast the CRISPR-based adaptive immunity system in bacteria with our own adaptive immunity system.

How do you think the CRISPR-Cas9 endonuclease might be engineered as a tool in editing the human genome.