

CRISPR: Introducing a New Era in Molecular Biology

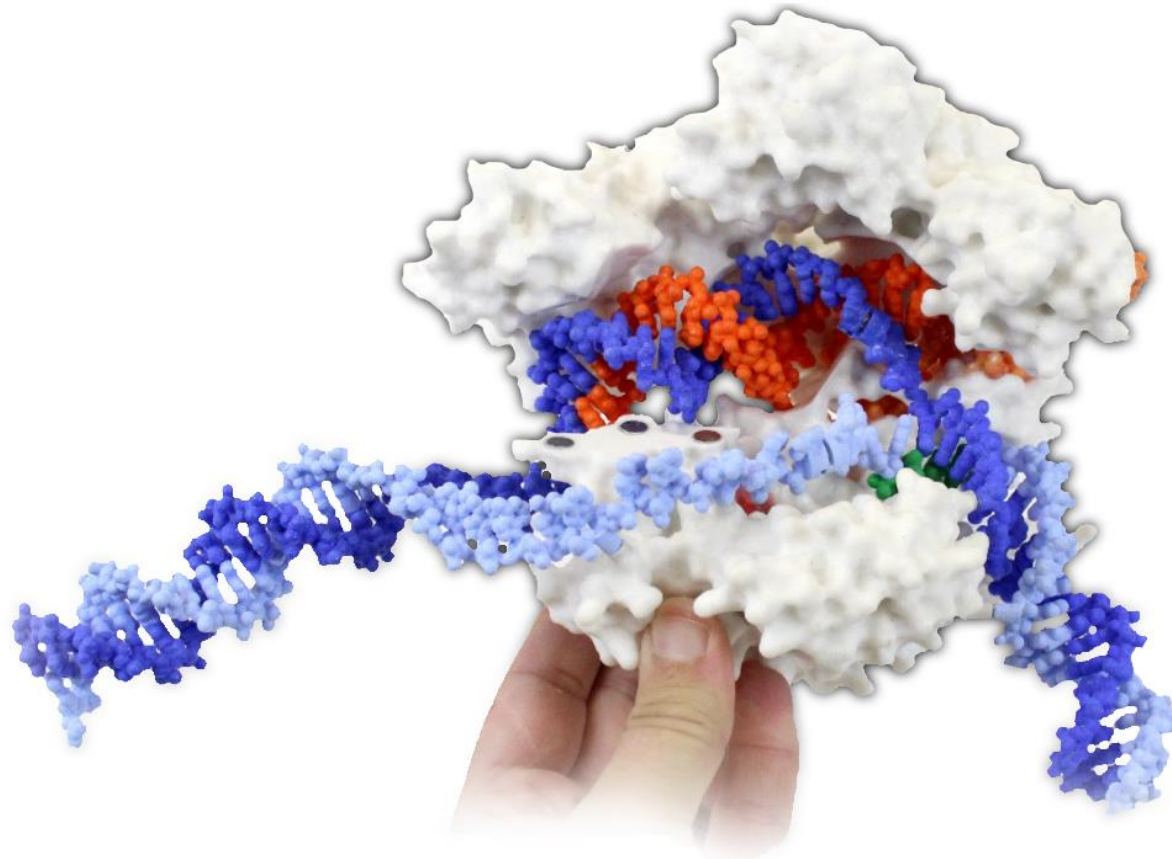
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The Science and Ethics of CRISPR-based Genome Editing



CRISPR *as a story of the process of science*



<https://www.youtube.com/watch?v=KNyFIXkszkM>

Should we introduce high school students to CRISPR?

YES,...because....:

1. CRISPR has revolutionized how molecular science is done.
2. CRISPR technology will be used to edit eukaryotic genomes.
3. Important ethical issues related to the use of CRISPR technology exist.



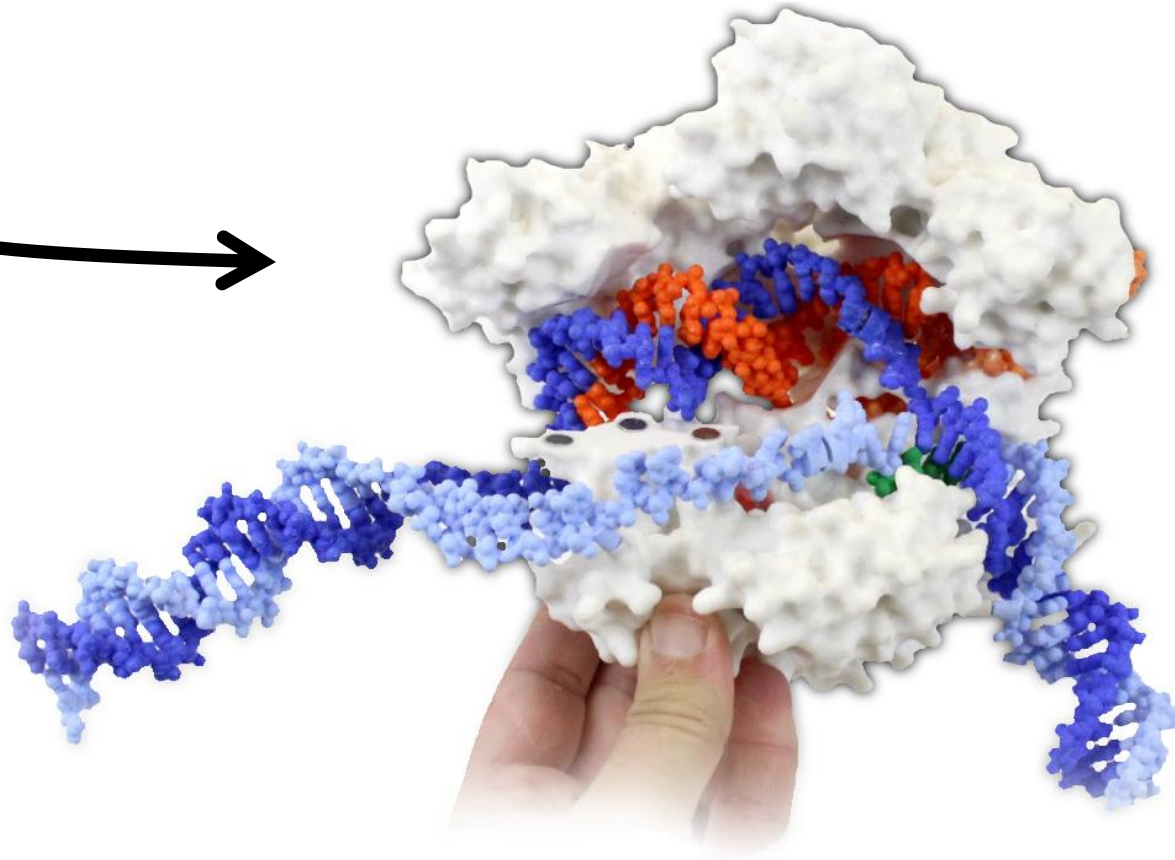
4. **CRISPR** is an incredible story of *the process of science*.

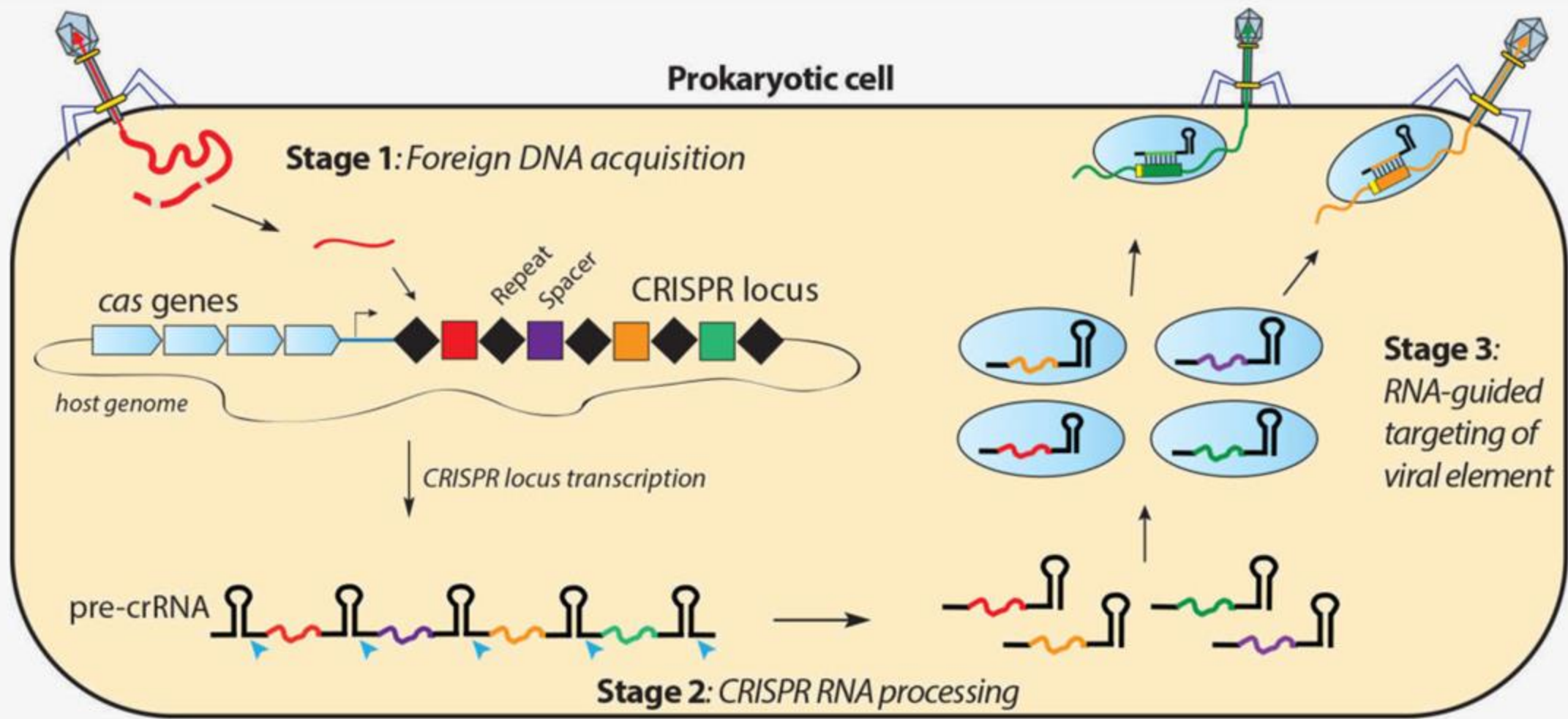
So,... how should we introduce students to CRISPR?

- *How can we capture students' interest in CRISPR?*
- *How can we connect CRISPR to what we are already teaching?*

But first,...**CRISPR**... *as an adaptive immunity system in bacteria.*

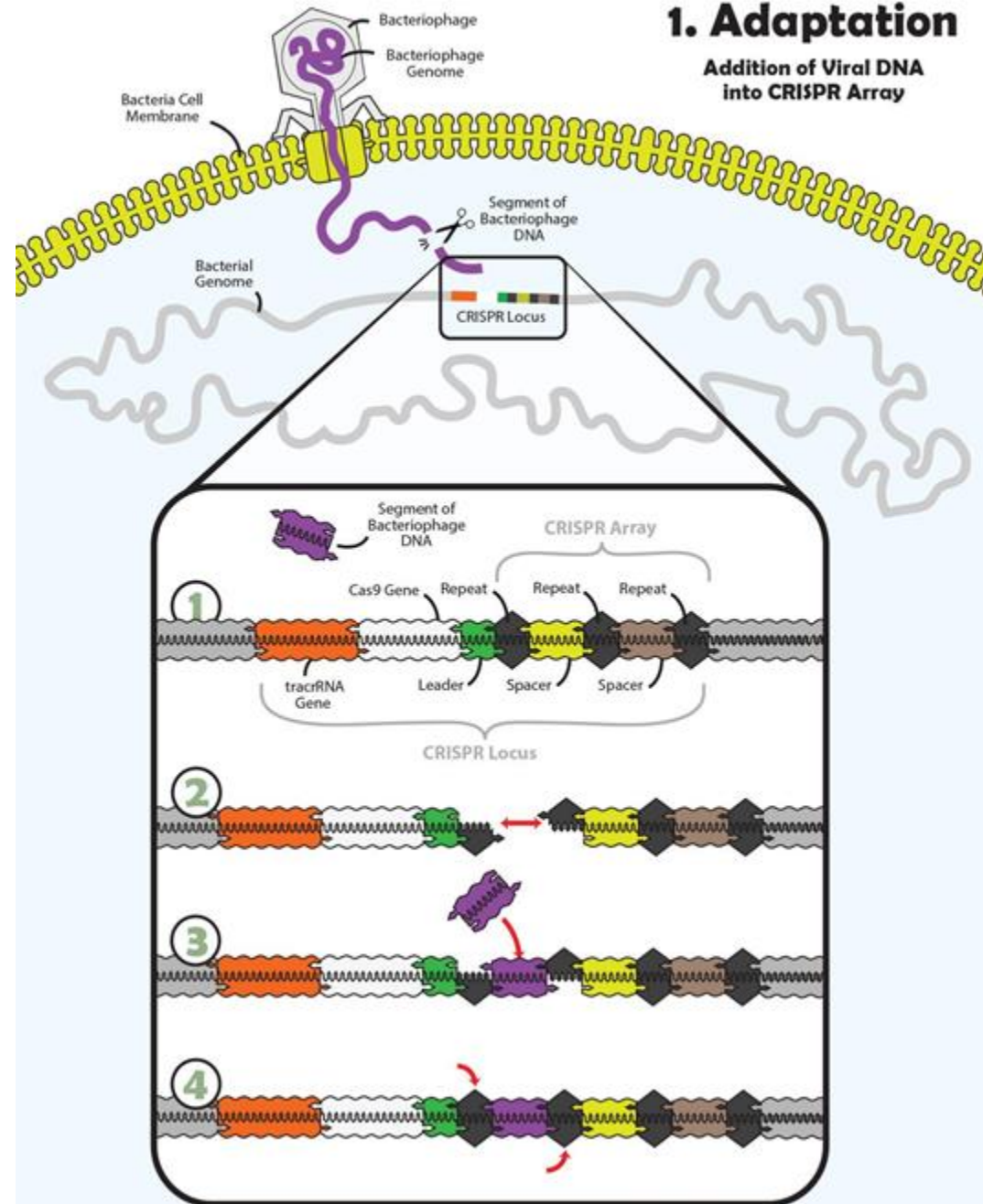
CRISPR Cas9





CRISPR – **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

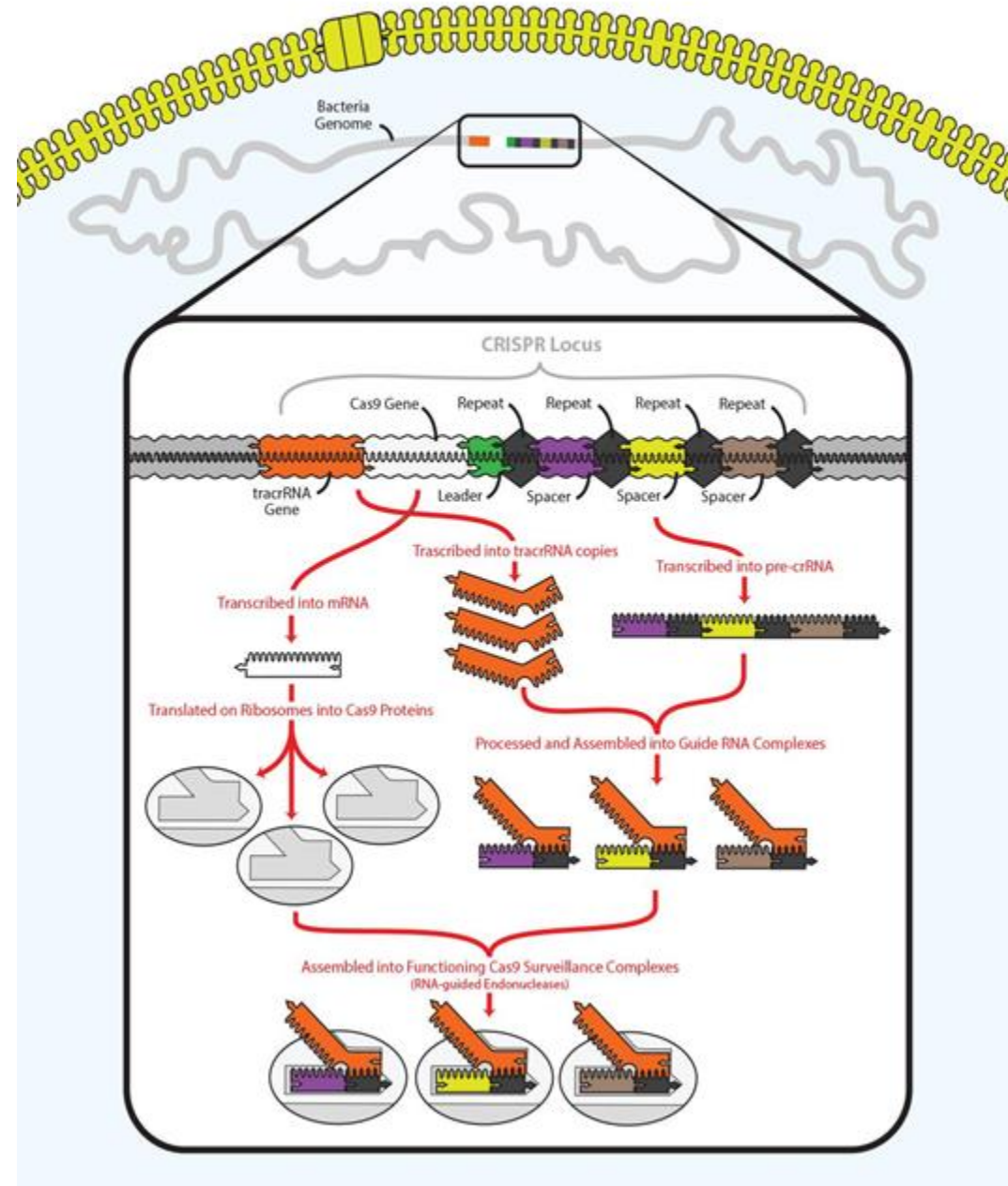
3 Guiding Graphics



3 Guiding Graphics

2. Expression of CRISPR Locus Genes

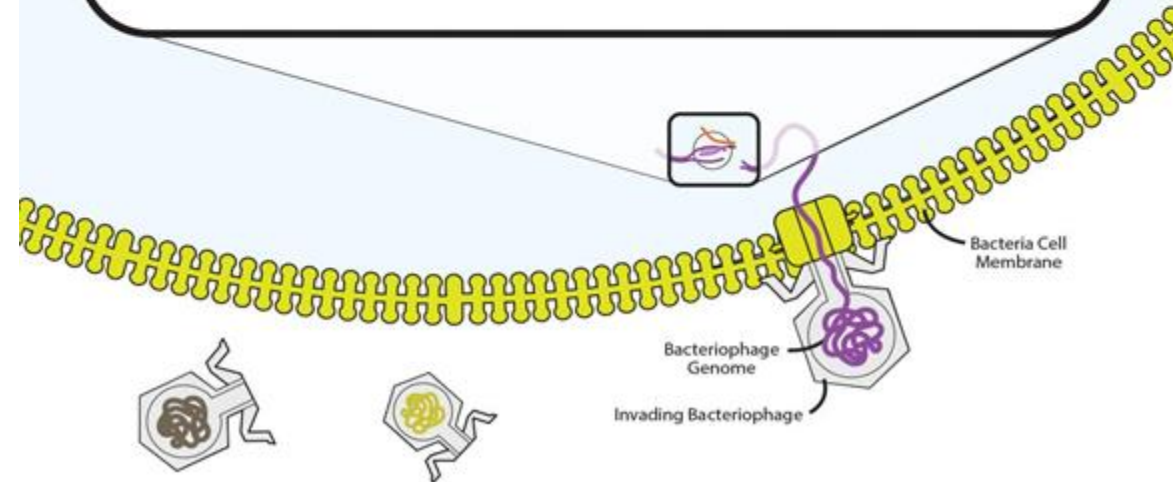
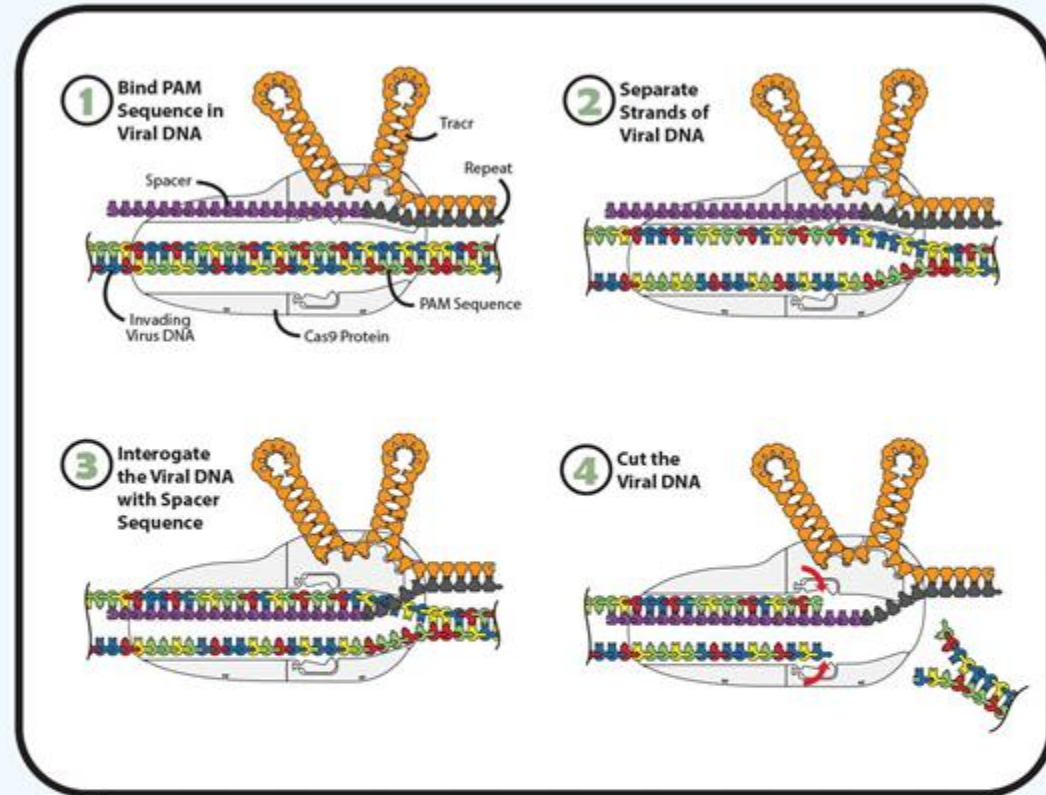
Building Cas9 Surveillance Complexes



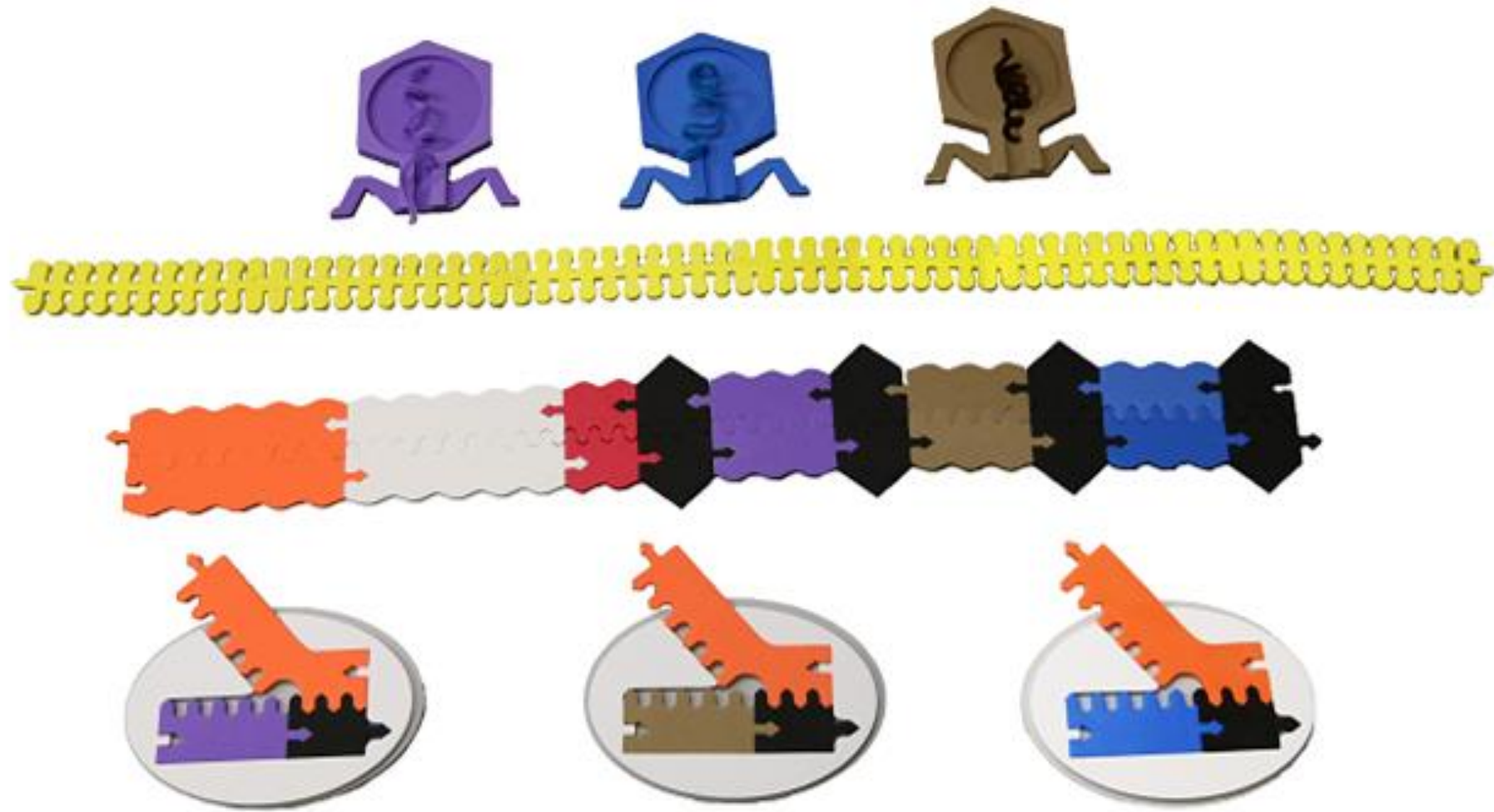
3 Guiding Graphics

3. Invader Silencing

Cutting the DNA of an Incoming Virus

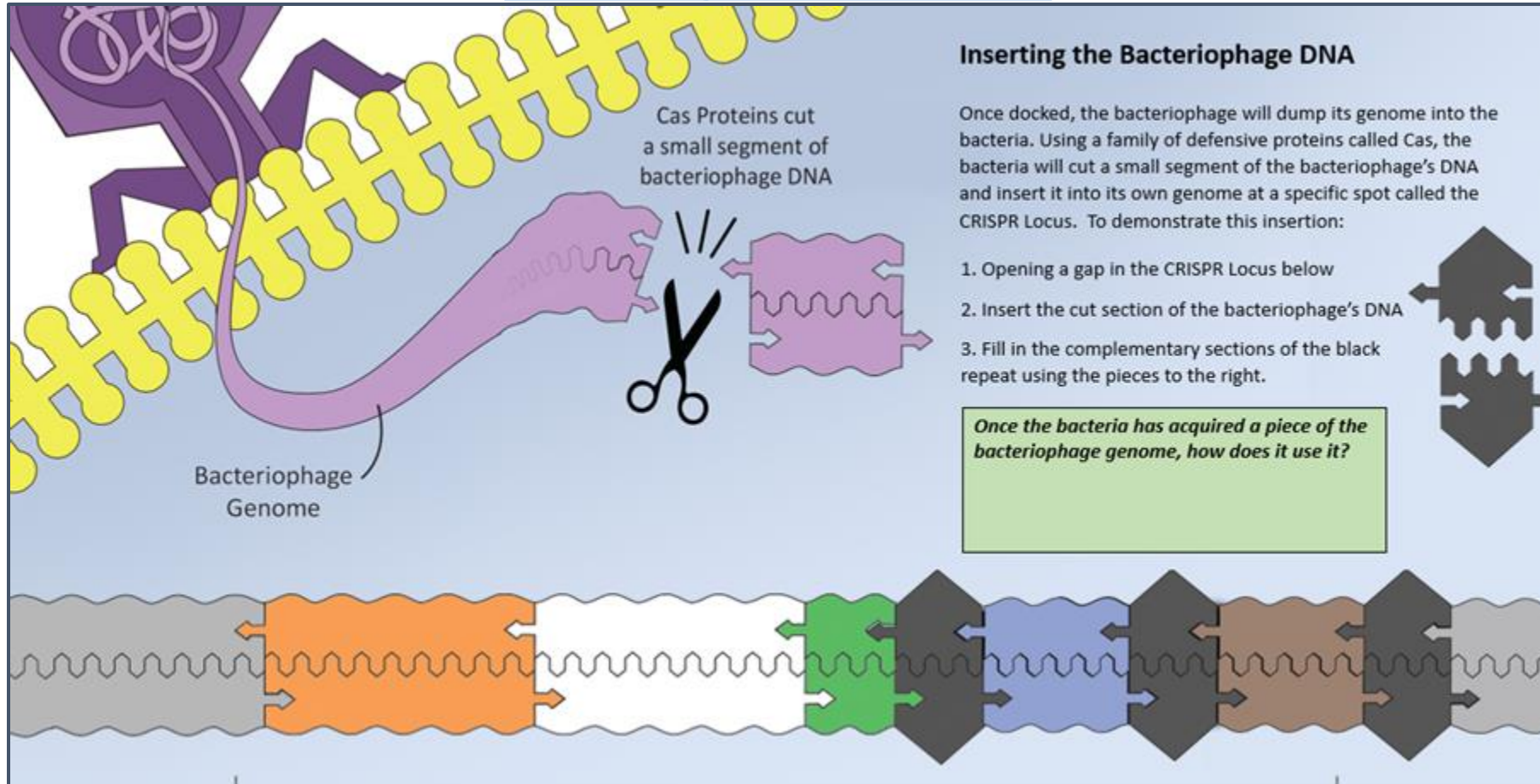


CRISPR Adaptive Immunity Kit Teacher Resources



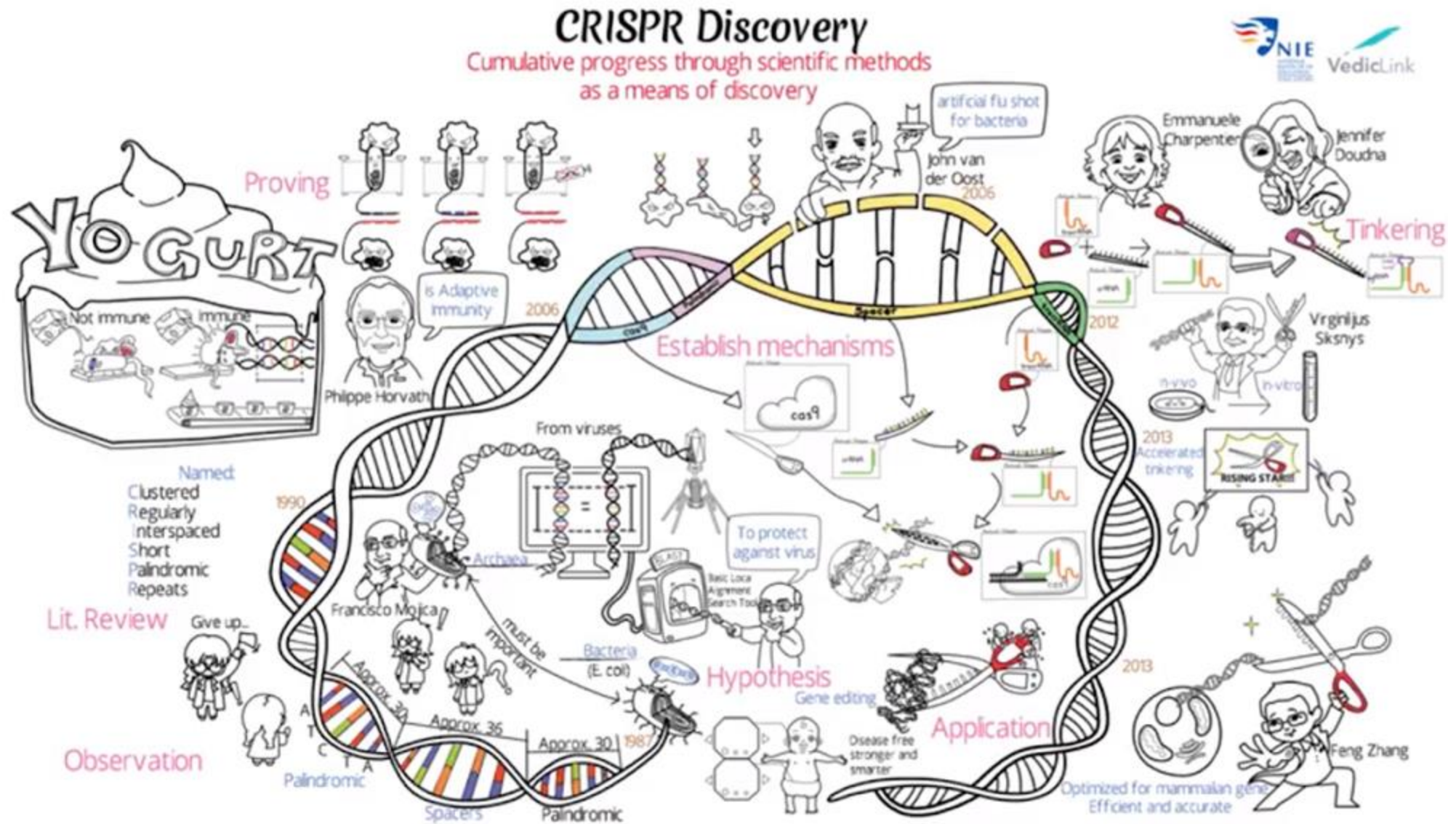
Interactive Digital Activities

<https://www.3dmoleculardesigns.com/Teacher-Resources/CRISPR-Adaptive-Immunity-Kit/Digital-Student-Modeling-Activities.htm>



~~**Who discovered CRISPR?**~~

How was CRISPR discovered?



CRISPR: History of Discovery

<https://www.youtube.com/watch?v=RKh2mi3tsmc>

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

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Received 1 May 1987/Accepted 22 August 1987

The *iap* gene in *Escherichia coli* is responsible for the isozyme conversion of alkaline phosphatase. We analyzed the 1,664-nucleotide sequence of a chromosomal DNA segment that contained the *iap* gene and its flanking regions. The predicted *iap* product contained 345 amino acids with an estimated molecular weight of 37,919. The 24-amino-acid sequence at the amino terminus showed features characteristic of a signal peptide. Two proteins of different sizes were identified by the maxicell method, one corresponding to the Iap protein and the other corresponding to the processed product without the signal peptide. Neither the isozyme-converting activity nor labeled Iap proteins were detected in the osmotic-shock fluid of cells carrying a multicopy *iap* plasmid. The Iap protein seems to be associated with the membrane.

In the last paragraph of the Discussion.....

An unusual structure was found in the 3'-end flanking region of *iap* (Fig. 5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing. The first sequence was included in the putative transcriptional termination site and had less homology than the others. Well-conserved nucleotide sequences containing a dyad symmetry, named REP sequences, have been found in *E. coli* and *Salmonella typhimurium* (28) and may act to stabilize mRNA (18). A dyad symmetry with 14 nucleotide pairs was also found in the middle of these sequences (underlining, Fig. 5), but no homology was found between these sequences and the REP sequence. So far, no sequence homologous to these has been found elsewhere in procaryotes, and the biological significance of these sequences is not known.

This is how science often happens.....

Could you have discovered CRISPR?

(If you had been working alongside Yoshizumi Ishino in 1987,... would you have noticed the unusual pattern in the nucleotide sequence downstream of the IAP gene?)

**GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGT
GCGCCCGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCA
CCGTCGTTGCCGGTTTATCCCTGCTGGCGCGGGGGAACTCTCGGTTCAGGCGTTGCA
AACCTGGCTACCGGGCGGTTTATCCCCGCTAACGCGGGGGAACTCGTAGTCCATCAT
TCCACCTATGTCTGAACTCCCGGTTTATCCCCGCTGGCGCGGGGGAACTCG**

Could you have discovered CRISPR?

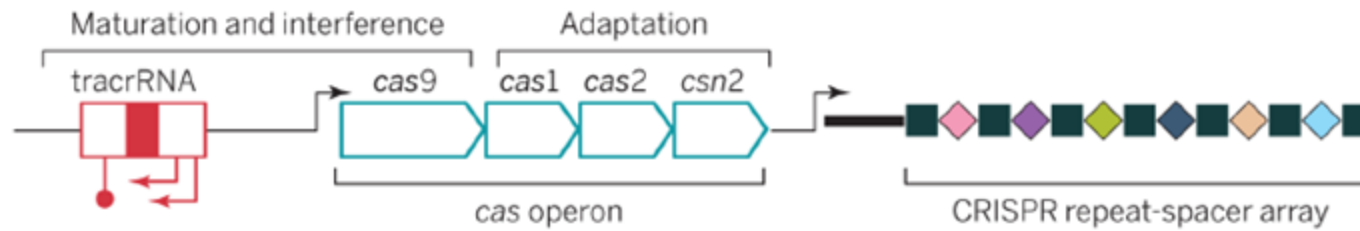
GGAGTTCACCGCAGAGGCGGGGGAAC TCCAAGTGATATCCATCATCGCATCCAG
TGCGCCCGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCT
CACCGTCGTGCGGTTTATCCCTGCTGGCGCGGGGAAC TCTCGGTT CAGGCGTT
GCAAACCTGGCTACCGGGCGGTTTATCCCCGCTAACGCGGGGAAC TCGTAGTCCA
TCATTCCACCTATGTC TGAAC TCCCGGTTTATCCCCGCTGGCGCGGGGAAC TCG

Could you have discovered CRISPR?

GGAGTTCACCGCAGAGGCGGGGGAAC**TCCAAGTGATATCCATCATCGCATCCAGTGCGCC**
CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
CGGTTTATCCCTGCTGGCGCGGGGAAC**TC**TCGGTTCAGGCGTTGCAAACTGGCTACCGGG
CGGTTTATCCCCGCTAACGCGGGGAAC**TCGTAGTCCATCATTCCACCTATGTC**TGAAC**TCC**
CGGTTTATCCCCGCTGGCGCGGGGAAC**TCG**

29 nucleotide
“Short Palindromic Repeat”

32 nucleotide
“interspersed spacer”



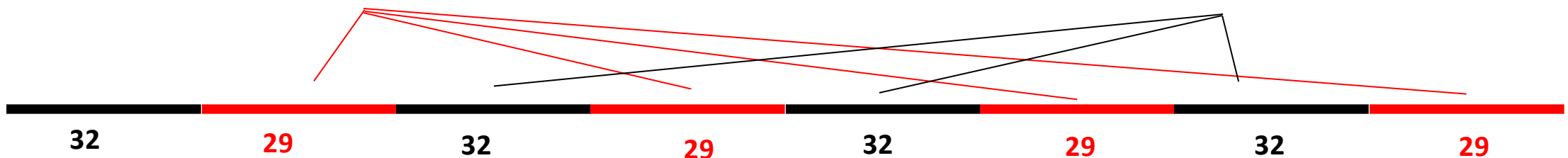
GGAGTTCTACCGCAGAGGCGGGGGAACCTCAAGTGATATCCATCATCGCATCCAGTGCGCC
 CGGTTTATCCCCGCTGATGCGGGGAACACAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
 CGGTTTATCCCTGCTGGCGCGGGGGAACCTCGGTTTCAGGCGTTGCAAACCTGGCTACCGGG
 CGGTTTATCCCCGCTAACGCGGGGGAACCTCGTAGTCCATCATTCCACCTATGTCTGAACCTCC
 CGGTTTATCCCCGCTGGCGCGGGGGAACCTCG

CRISPR repeats (29)

Clustered Regularly Interspersed Palindromic Repeats

Spacer sequences (32)

(later shown to be foreign DNA)



Could you have discovered CRISPR?

The Ishino Sequences are available at:

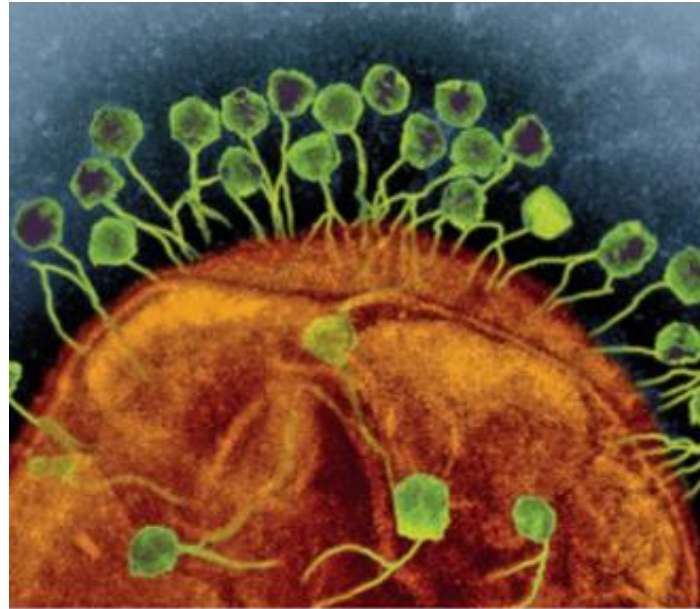
Black & White Version: <https://cbm.msoe.edu/markMyweb/crisprSequence1.html>

Color Version: <https://cbm.msoe.edu/markMyweb/crisprSequence2.html>

MICROBIOLOGY

Microbial warfare against viruses

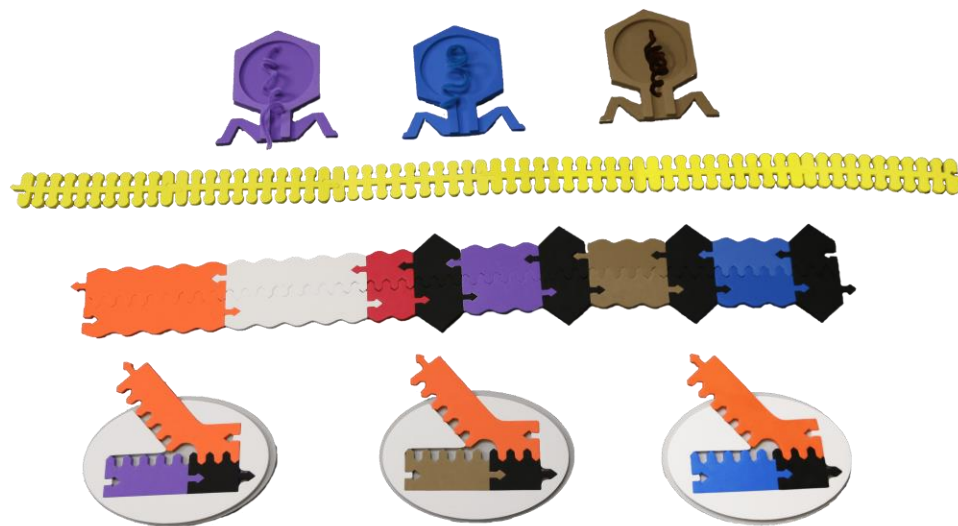
Many new antiviral defense systems are found in bacteria and archaea



An electron microscopy image shows viruses (bacteriophage) surrounding a microbial cell.

THANK YOU FOR ATTENDING!

Models and posters used in this session:



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