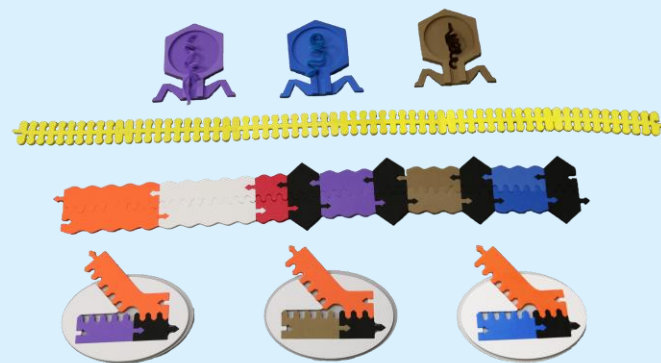
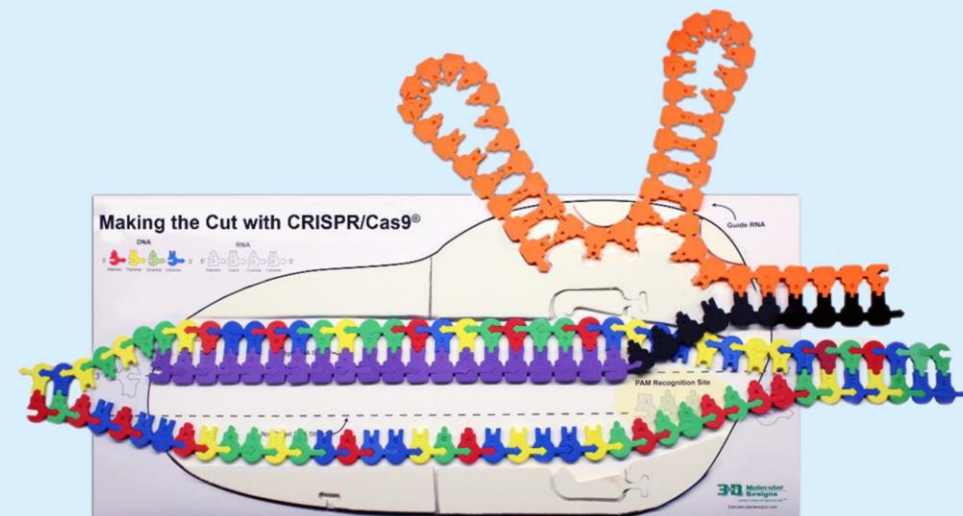




CRISPR: Isn't it Time We Fixed Your Genome?



Tim Herman, PhD.
Founding Partner & Science Officer



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?*

CRISPR...

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

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?)

GGAGTTCTACCGCAGAGGCGGGGGGAAGTCCAAGTGATATCCATCATCGCATCCAGT
GCGCCCGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCA
CCGTCGTTGCCGGTTTATCCCTGCTGGCGCGGGGAAGTCTCGGTTTCAGGCGTTGCA
AACCTGGCTACCGGGCGGTTTATCCCCGCTAACGCGGGGAAGTCGTAGTCCATCAT
TCCACCTATGTCTGAAGTCCCGGTTTATCCCCGCTGGCGCGGGGAAGTCG

Could you have discovered CRISPR?

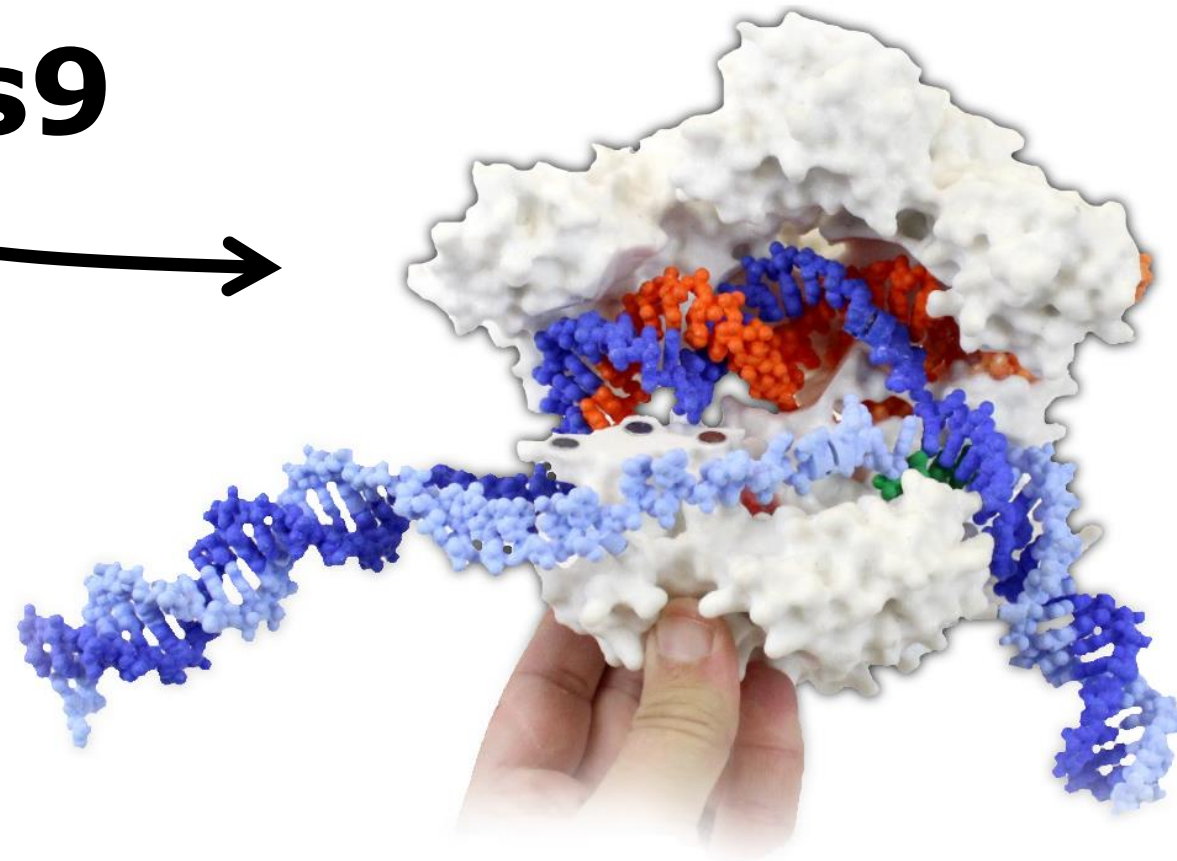
GGAGTTCACCGCAGAGGCGGGGGAAC TCCAAGTGATATCCATCATCGCATCCAGTGC GCC
CGGTTTATCCCCGCTGATGCGGGGAAC ACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
CGGTTTATCCCTGCTGGCGCGGGGGAAC TCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG
CGGTTTATCCCCGCTAACGCGGGGAAC TCGTAGTCCATCATTCCACCTATGTC TGAAC TCC
CGGTTTATCCCCGCTGGCGCGGGGGAAC TCG

Short Palindromic Repeats
(30 nucleotides)

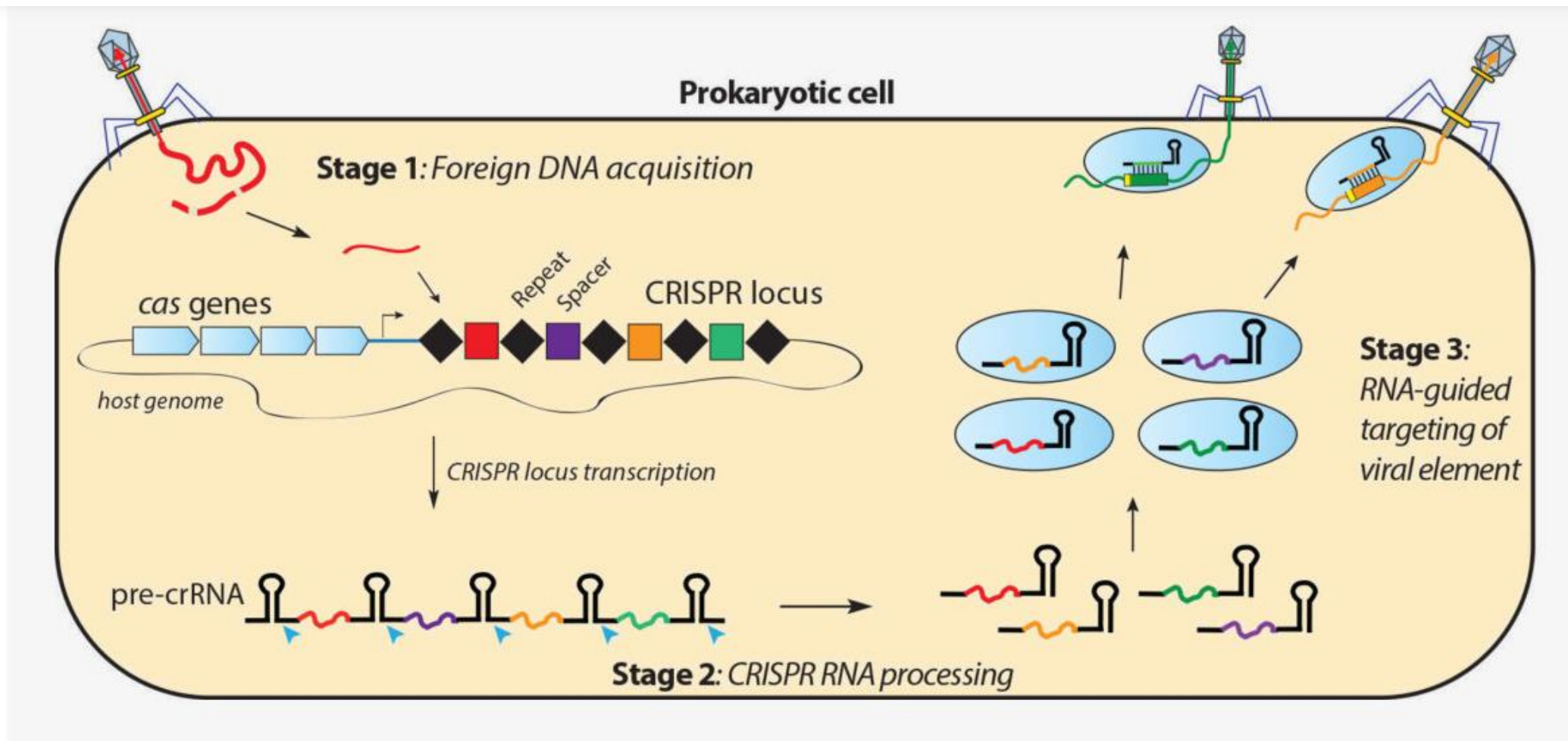
Spacer Sequences
(bacteriophage sequences)
(32 nucleotides)

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

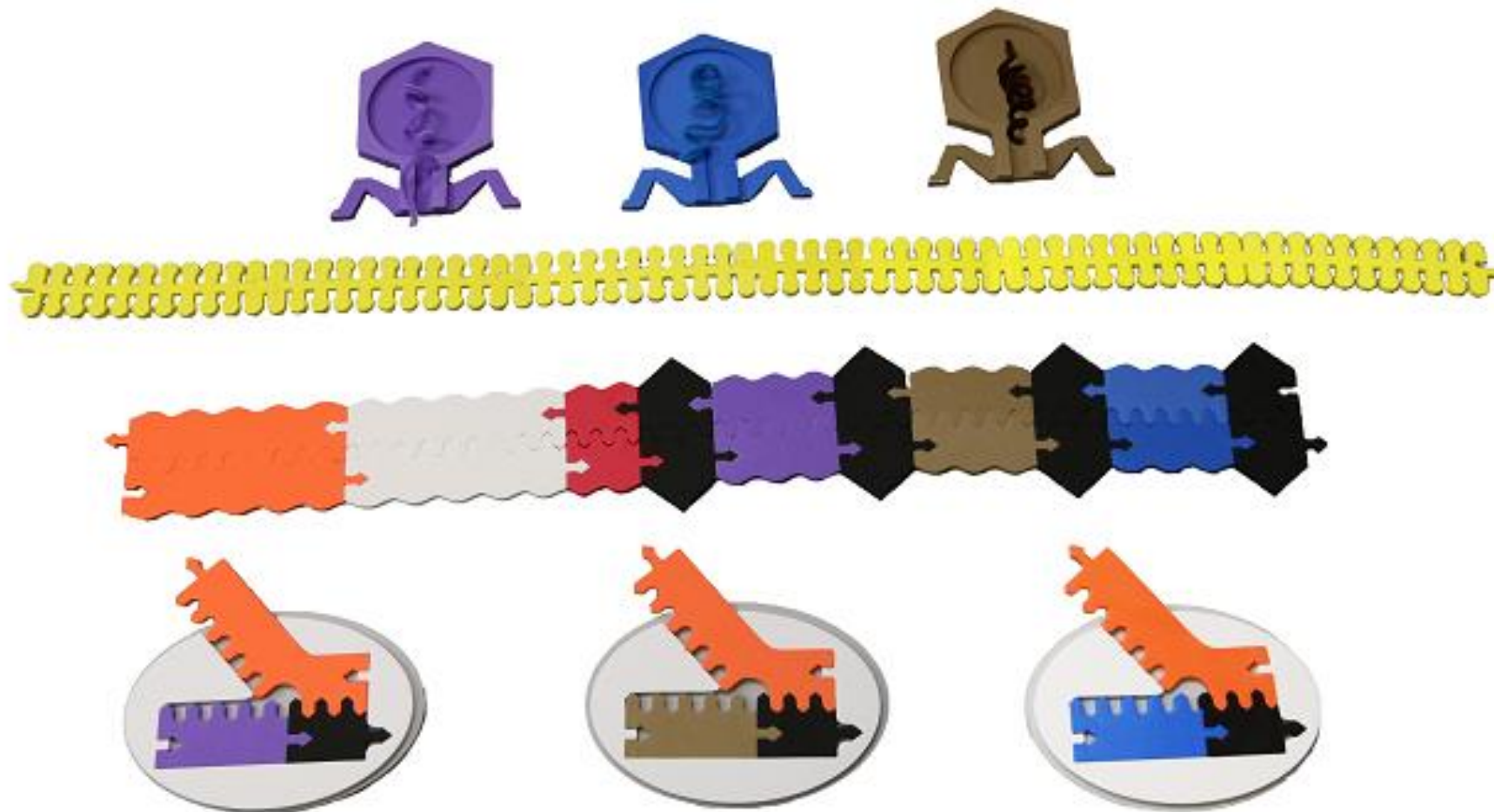
CRISPR Cas9



CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats

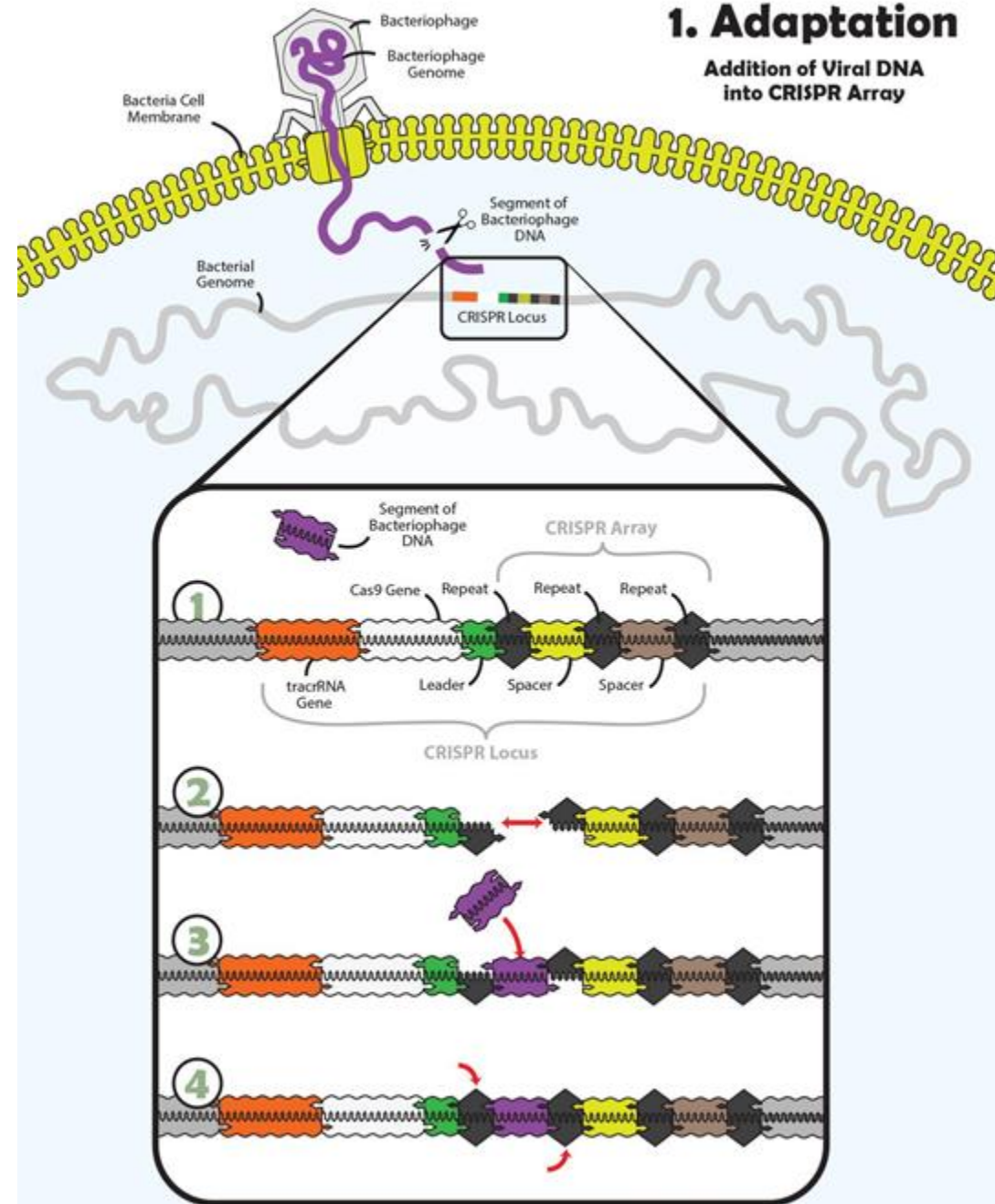


CRISPR Adaptive Immunity Kit Teacher Resources



[Teacher Resources \(3dmoleculardesigns.com\)](http://3dmoleculardesigns.com)

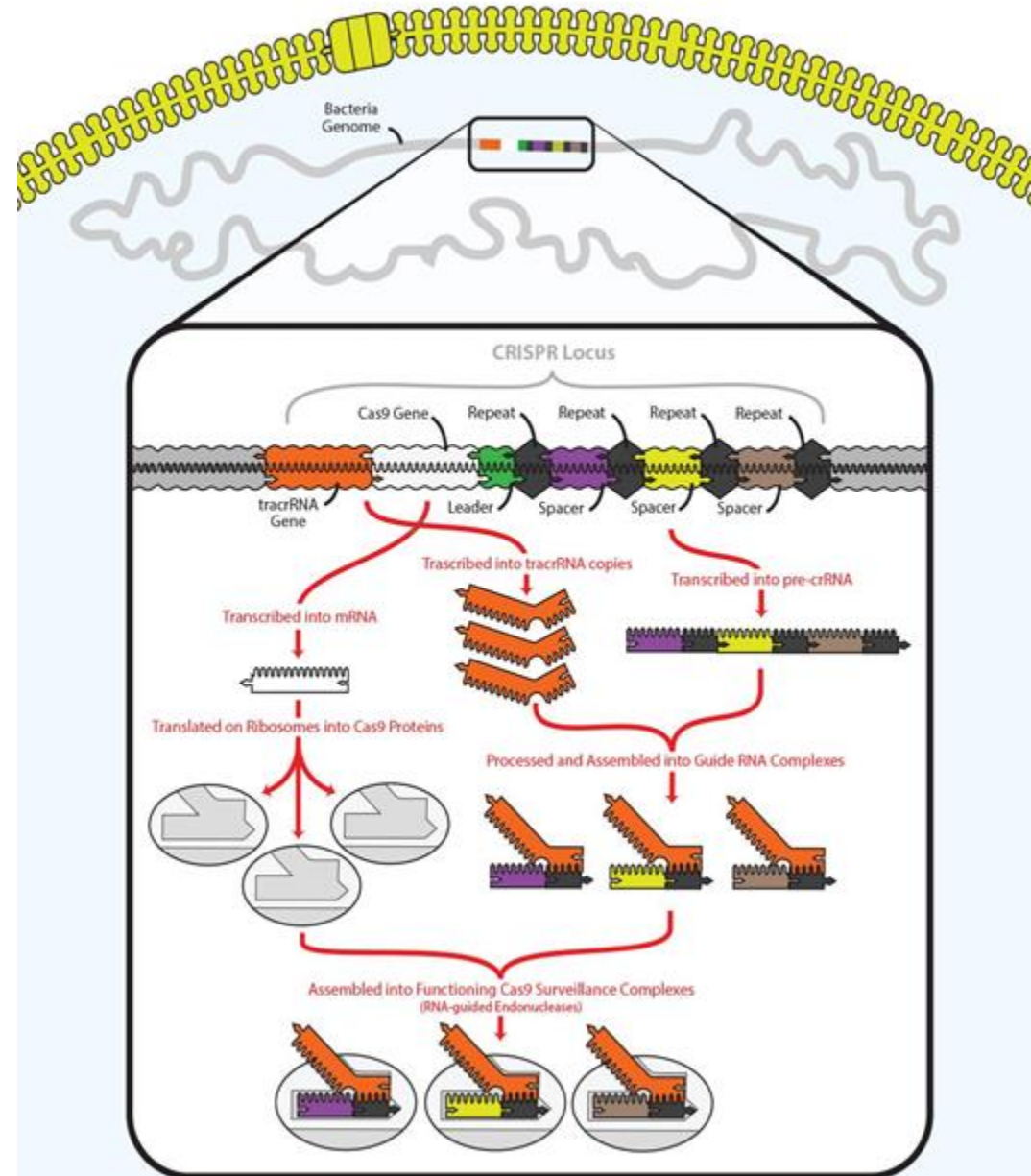
Guiding Graphics

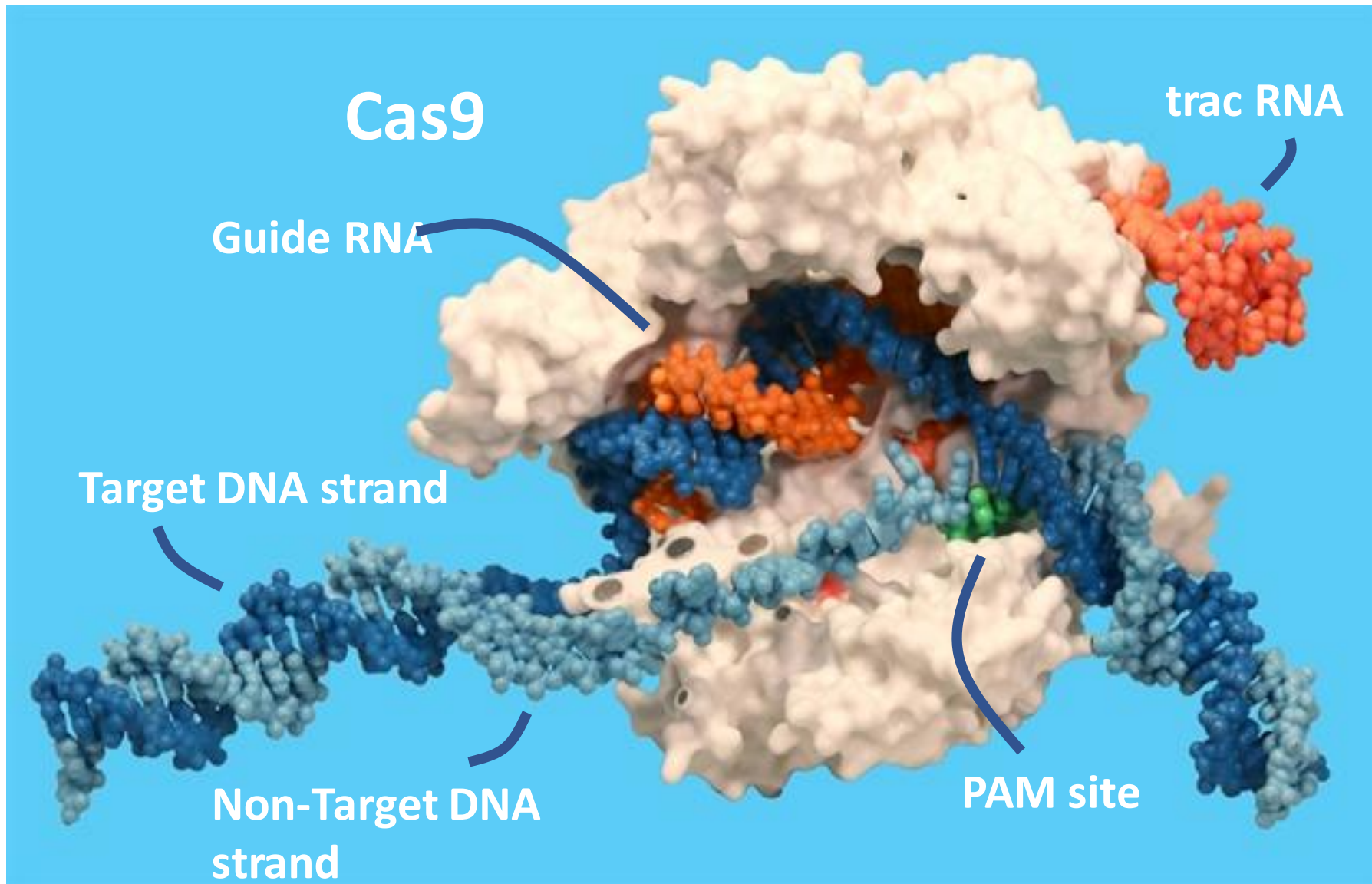


Guiding Graphics

2. Expression of CRISPR Locus Genes

Building Cas9 Surveillance Complexes





Jennifer Doudna



Image: Drew Kelly



Martin Jinek



Image: Philipp Rohner



The magic of models....

Models make words meaningful!

Interactive physical models provide students with a way to understand a description (written or verbal) of a complex molecular process – such as how CRISPR functions as an adaptive immunity system in bacteria.



A **Framework** for thinking about CRISPR

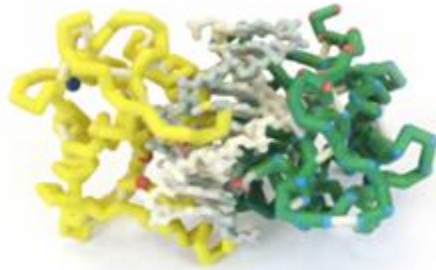
- **CRISPR Biology** ... *how does CRISPR function in bacteria as an adaptive immune system?*
- **CRISPR Biotechnology** ... *how are we using CRISPR tools to edit eukaryotic genomes?*

What makes Cas9 special?

*Cas9 can target a **statistically unique site** in the 3.2 billion base-pair human genome,*

...and make a double-stranded cut.

The Evolution of Genome Engineering Technology



Restriction Endonucleases

In the 1970's, restriction endonucleases were the enabling technology that led to genetic engineering. They recognized 6-8 nucleotide-long sequences, and cut the human genome into thousands of small fragments.



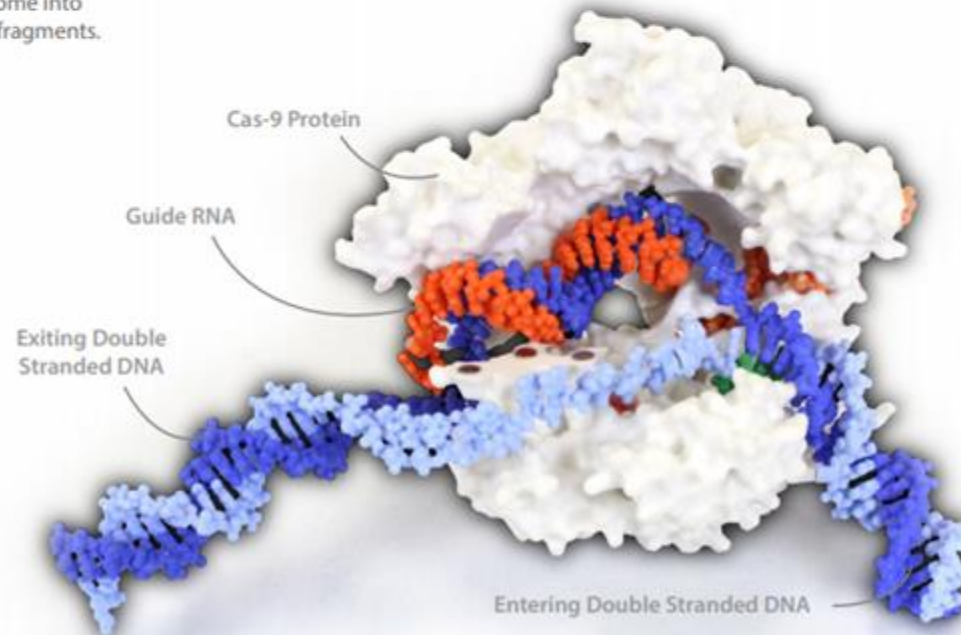
Zinc Finger Nuclease

In the 1980's, zinc finger proteins were recognized as specific DNA binding motifs. Researchers soon learned how to engineer the amino acid sequence of the fingers to direct them to different 3-bp target sequences. In subsequent work, multiple engineered fingers were strung together (polydactile zinc fingers), fused with nuclease domains, and used to introduce double-stranded cuts at unique sites in the human genome.



TALEN Proteins

From 2000 to 2010, the modular nature of TALENS was exploited to make them powerful gene editing agents.

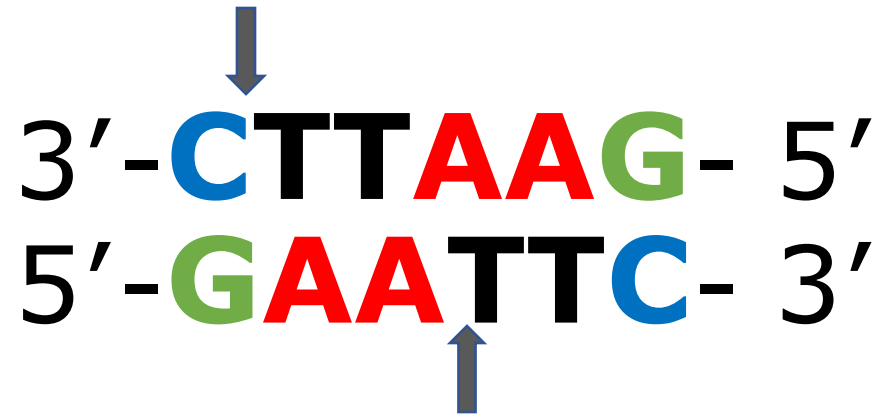


CRISPR Cas-9

In the early 2000's an adaptive immunity system in bacteria was discovered,...and by 2012 the utility of the CRISPR Cas9 endonuclease in engineering the human genome was demonstrated. CRISPR Cas9 is fundamentally different than the preceding proteins in the way it recognizes a specific DNA sequence. Instead of relying on specific protein-DNA interactions, the Cas9 protein carries a "guide RNA" – that forms standard Watson-Crick base pairs with the target DNA. The Cas9 endonuclease targets a 21 base pair sequence of DNA – a sufficiently long sequence to be statistically unique in the human genome.



EcoRI... a restriction enzyme that cuts DNA
at a 6 base-pair palindromic site:



In a random sequence of nucleotides, you would expect to find an *EcoRI* restriction site every 4096 nucleotides.

The human genome is made up of **3,200,000,000** bp of DNA.

Since EcoRI cuts every **4096** bp... EcoRI would cut the human genome into:

$$\frac{3,200,000,000}{4,096} = \underline{\underline{781,250 \text{ fragments}}}$$

in other words... a mess

**CRISPR Cas9 cuts the human genome only once
... at a statistically unique site.**

*How many nucleotides must Cas9 be able to
recognize...for it to be a statistically unique sequence in
the human genome?*

The answer is... 12 16 24 38 42 ?

A statistically unique site in the human genome...

G A A T T C G T C G C T A A T G

16 nucleotides

$$4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 \times 4 = \underline{4.3 \times 10^9}$$

...or...

$$4^{16} = \underline{4.3 \times 10^9}$$

CRISPR: Connecting New Science to What You Already Teach



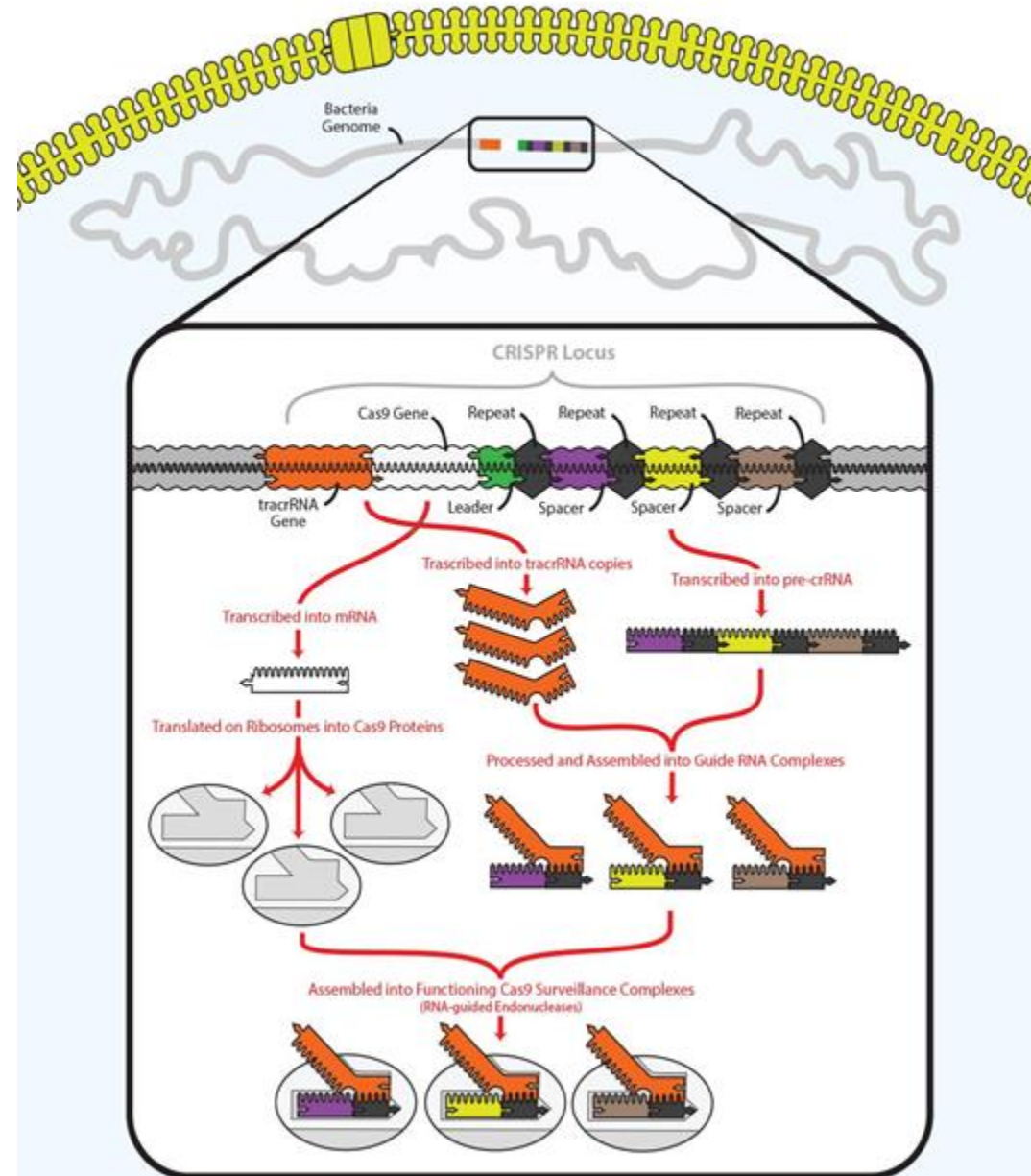
<https://www.3dmoleculardesigns.com/Teacher-Resources/Making-the-Cut-with-CRISPR-Cas9-Animations-and-Videos.htm>



Guiding Graphics

2. Expression of CRISPR Locus Genes

Building Cas9 Surveillance Complexes

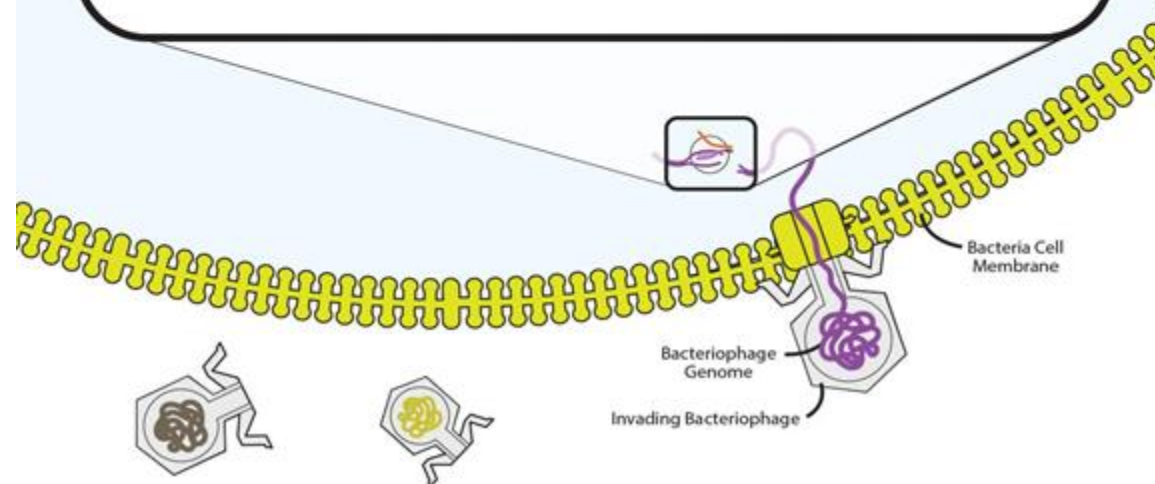
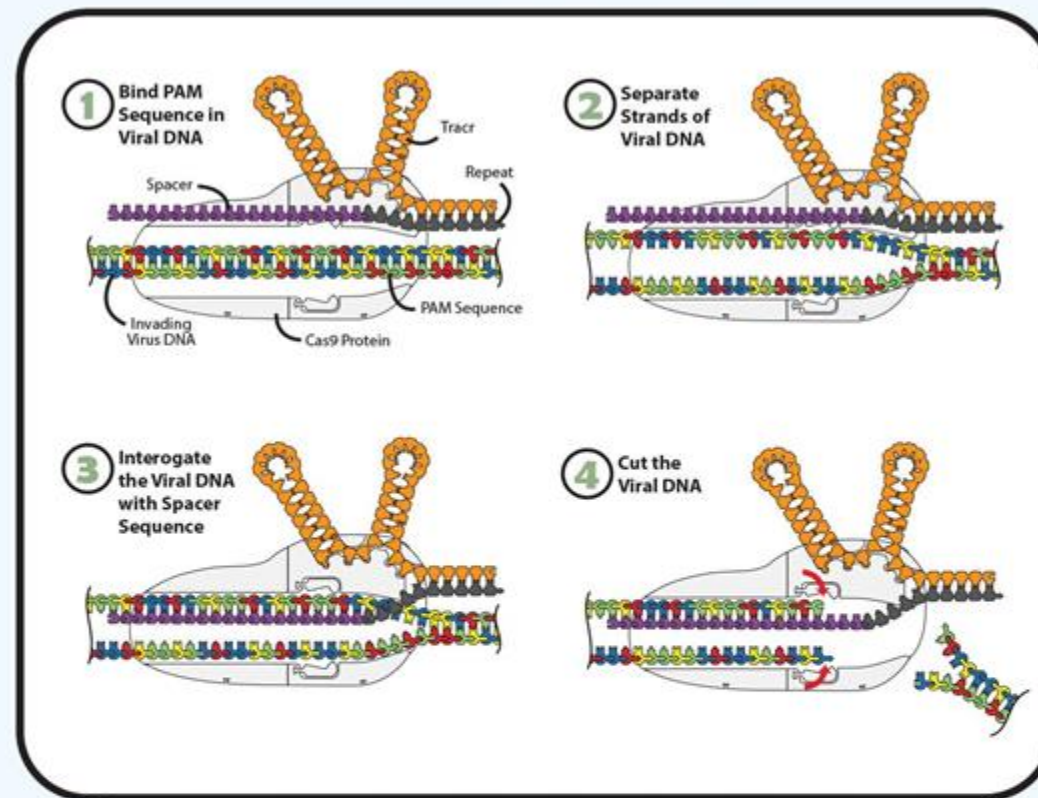




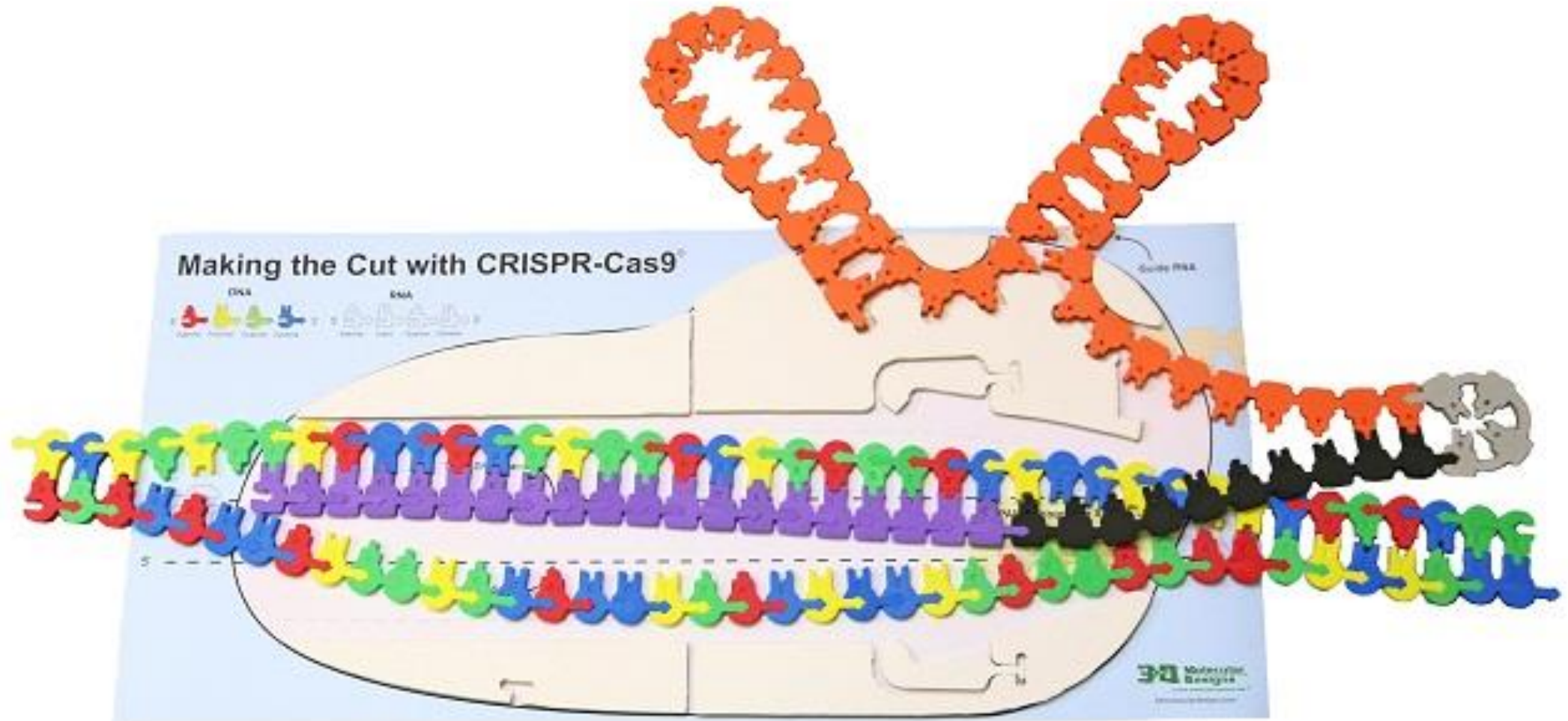
Guiding Graphics

3. Invader Silencing

Cutting the DNA of an Incoming Virus



The Making the Cut with the CRISPR-Cas9 Kit





5 Steps in the *Making the Cut*

Step 1... Assembling the tracrRNA...on the Cas9 protein

Step 2... Adding the crRNA to the tracr RNA

Step 3... Binding the PAM site of double-stranded DNA to Cas9

Step 4... Interrogating the Target DNA strand with the guide RNA

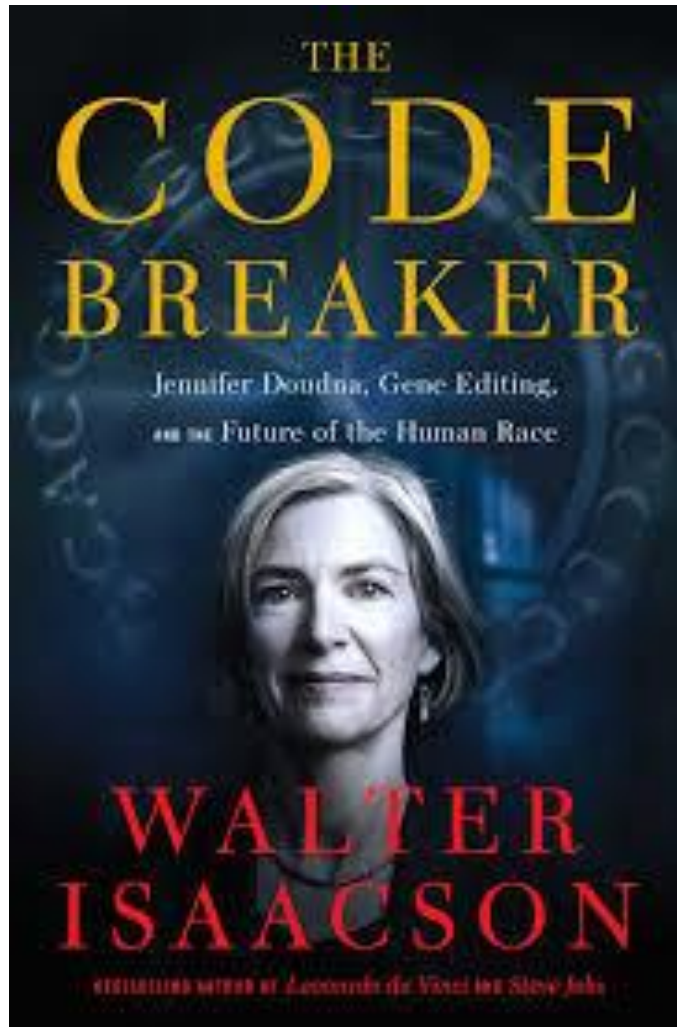
Step 5... Cutting the Target and Non-Target DNA strands



CRISPR Cas9... for real



Two must see / read accounts of the CRISPR story.



Two Cas9 Jmol Explorations...

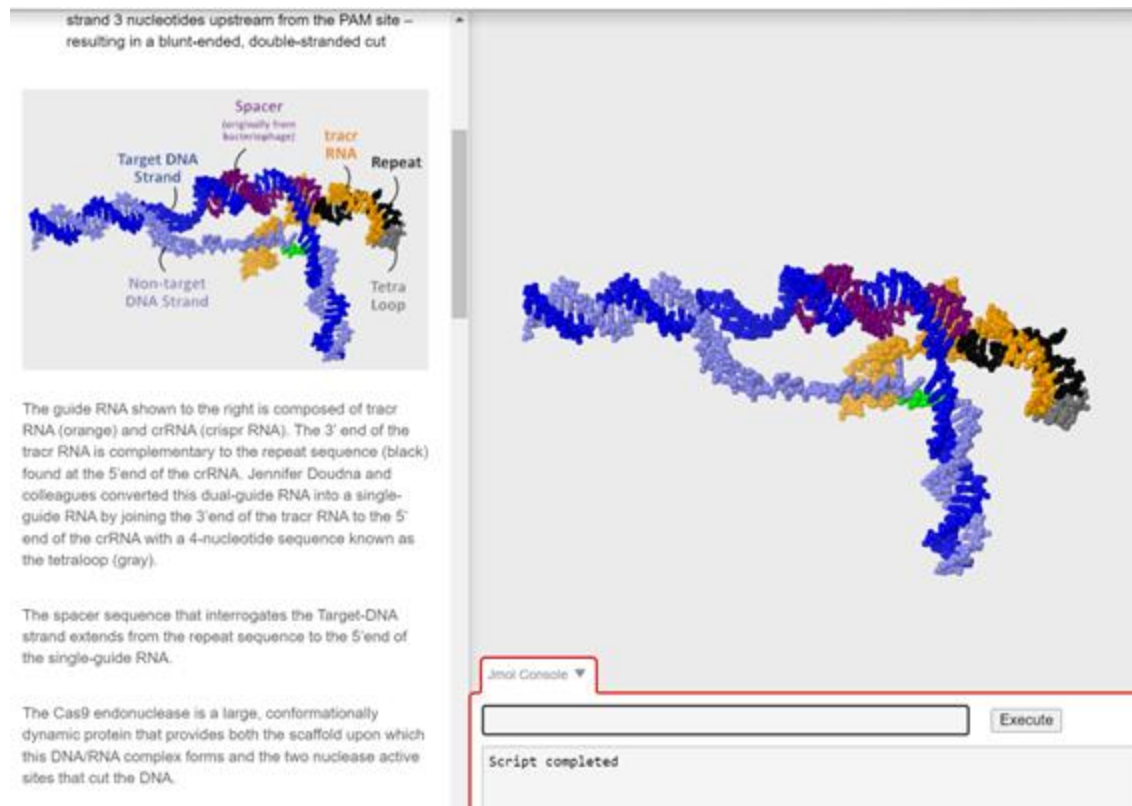
computer-based explorations of the structure of Cas9

1. Making the Cut with CRISPR Cas9

<https://www.centerforbiomolecularmodeling.org/markMyweb/basicPrinciplesOfChemistry/cas9.html>

1. The Conformationally Dynamic Domains of Cas9

<https://www.centerforbiomolecularmodeling.org/markMyweb/basicPrinciplesOfChemistry/timsCas9.html>

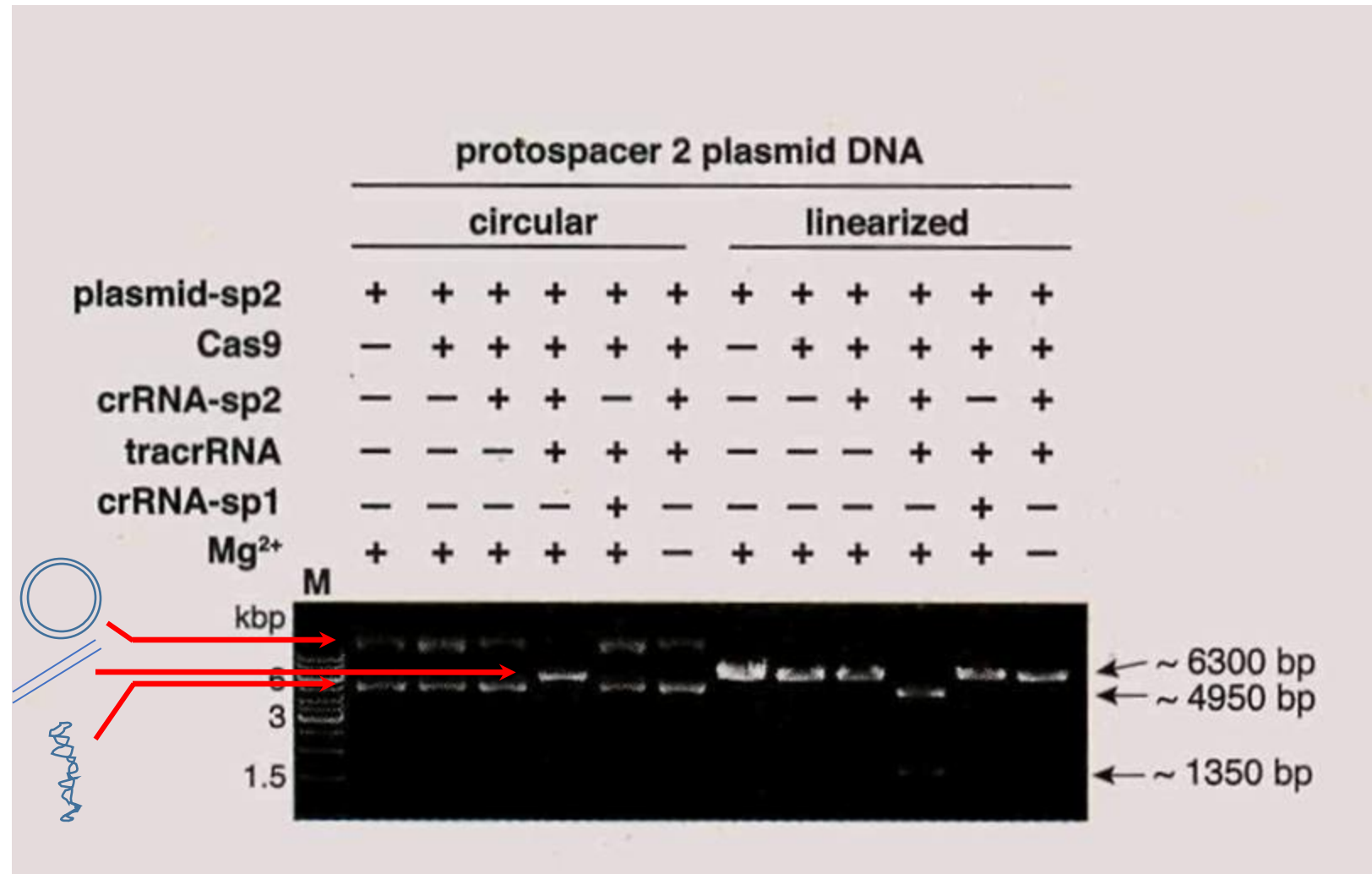


A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,^{1,2*} Krzysztof Chylinski,^{3,4*} Ines Fonfara,⁴ Michael Hauer,^{2†}
Jennifer A. Doudna,^{1,2,5,6‡} Emmanuelle Charpentier^{4‡}

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

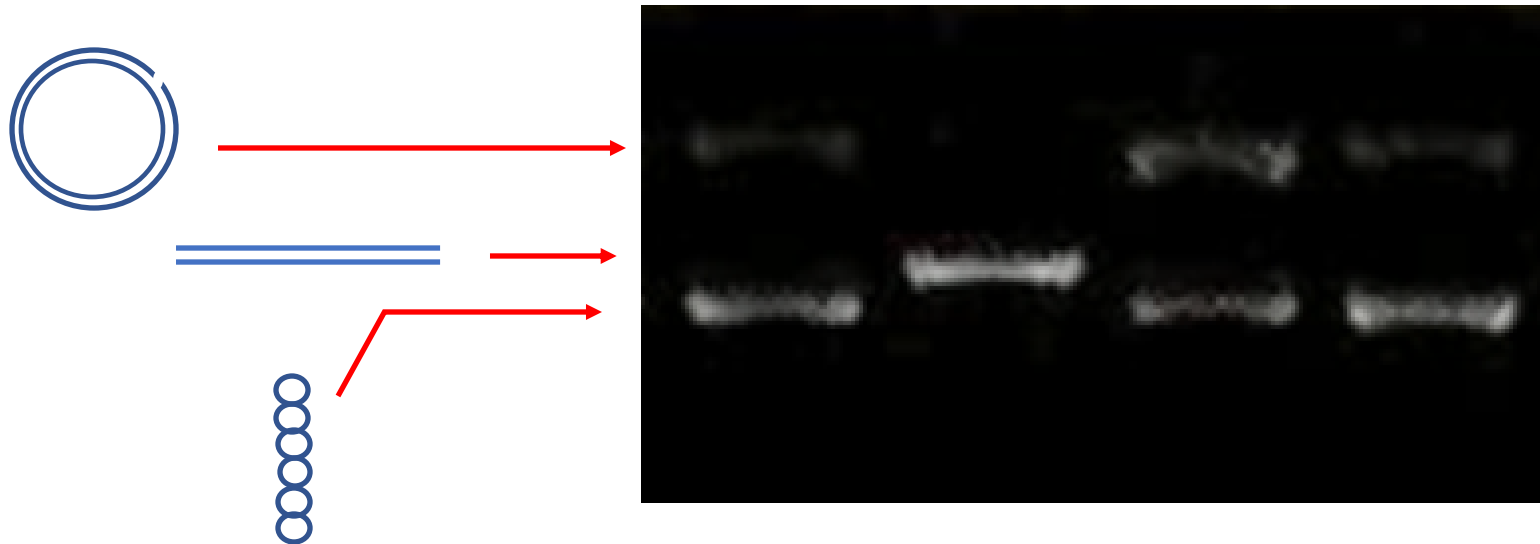
The “evidence” for much of what we know in the molecular biosciences is based on **shifts in bands on gels!**



Both tracrRNA and crRNA are necessary for Cas9 cleavage of DNA

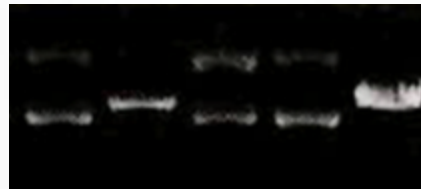
Experimental Evidence....that *tracRNA* was required for Cas9 endonuclease activity

Plasmid	+	+	+	+
Cas9	+	+	+	
-				
crRNA	+	+	-	+
tracRNA	-	+	+	+



Its all about

**Shifting
bands...**



**... on
gels !**



Concluding statement, 2012 Jinek paper

“We propose **an alternative methodology based on RNA-programmed Cas9** that could offer considerable potential for gene targeting and genome editing applications.”



What happens after the cut?

Minding our metaphors....

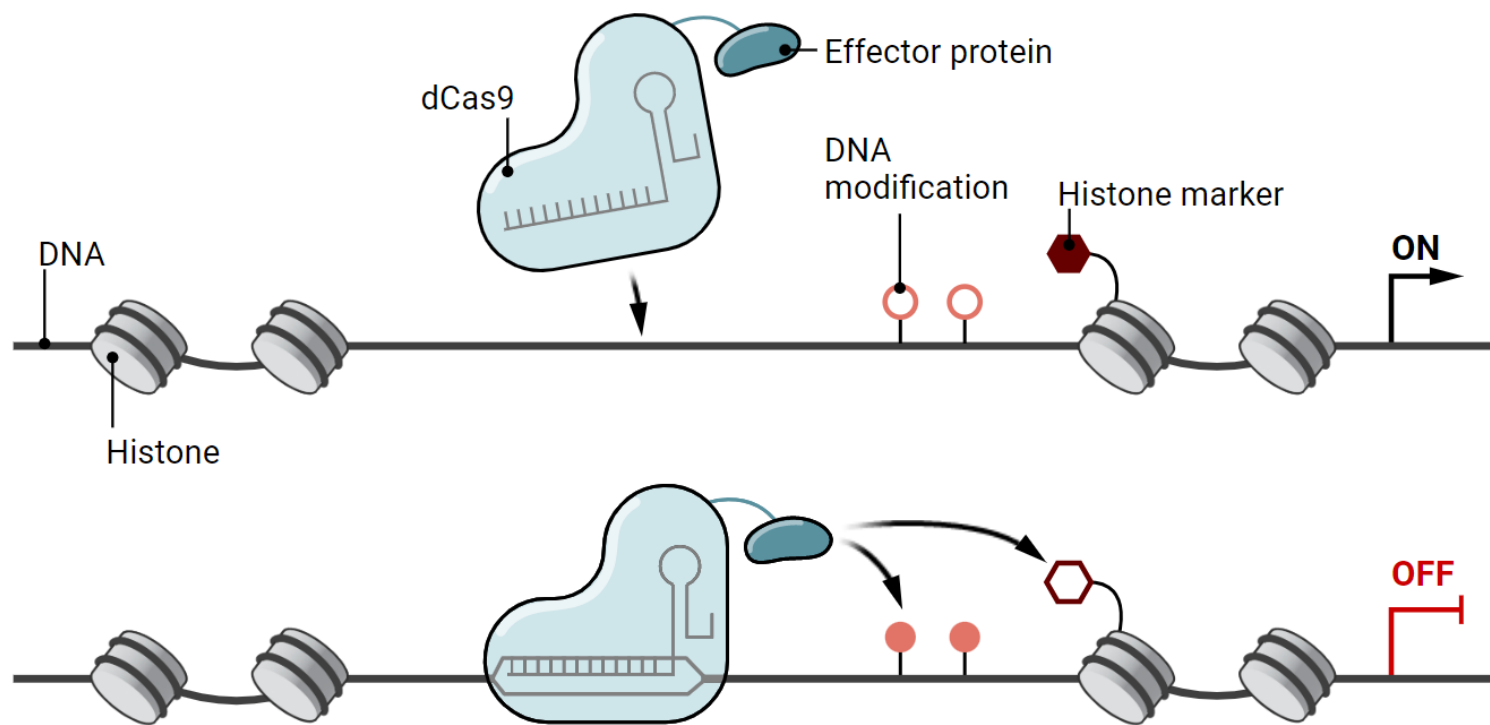
Stay tuned...



Regulation of Gene Expression and the Elucidative Role of CRISPR-Based Epigenetic Modifiers and CRISPR-Induced Chromosome Conformational Changes

Abstract

In complex multicellular systems, gene expression is regulated at multiple stages through interconnected complex molecular pathways and regulatory networks. Transcription is the first step in gene expression and is subject to multiple layers of regulation in which epigenetic mechanisms such as DNA methylation, histone tail modifications, and chromosomal conformation play an essential role. In recent years, CRISPR-Cas9 systems have been employed to unearth this complexity and provide new insights on the contribution of chromatin dysregulation in the development of genetic diseases, as well as new tools to prevent or reverse this dysregulation. In this review, we outline the recent development of a variety of CRISPR-based epigenetic editors for targeted DNA methylation/demethylation, histone modification, and three-dimensional DNA conformational change, highlighting their relative performance and impact on gene regulation. Finally, we provide insights on the future developments aimed to accelerate our understanding of the causal relationship between epigenetic marks, genome organization, and gene regulation.



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