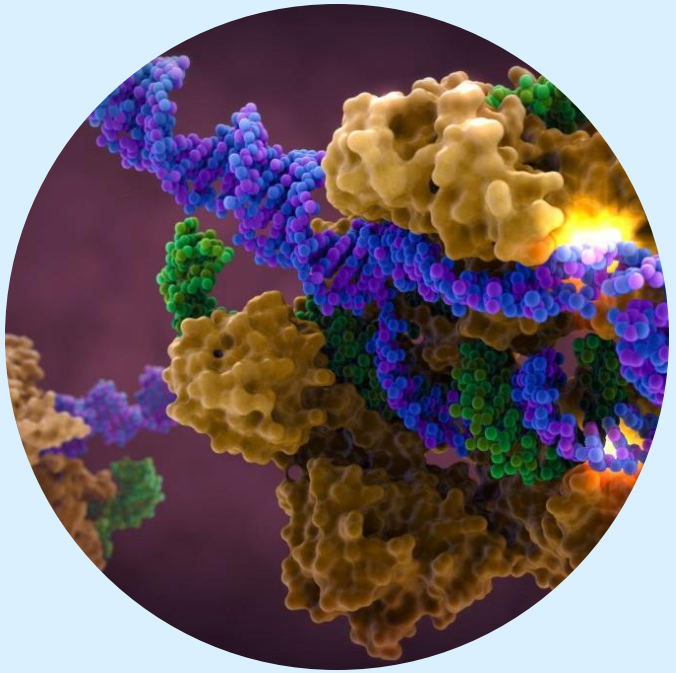
3d molecular designs
Transforming Science Learning

So effective, the viruses stole it!

CRISPR Cas-9 System
Structure and function

Mark Arnholt
Science Educator
3D Molecular Designs



Mark Arnholt

Science Educator

SMART Team Coordinator

3D Molecular Designs

Milwaukee, WI

mark.arnholt@3dmoleculardesigns.com

24 years of classroom experience

PLTW BMS Certified (PBS, MI, BI)
Cohort Biology
General Biology (9-10)
Anatomy and Physiology
Physical Science(9)
Aviation

Awards:

Outstanding Science Educator - University of Minnesota – Twin Cities
2014
Educator of the year HUHS
2015
Herb Kohl Fellowship Grant
2016

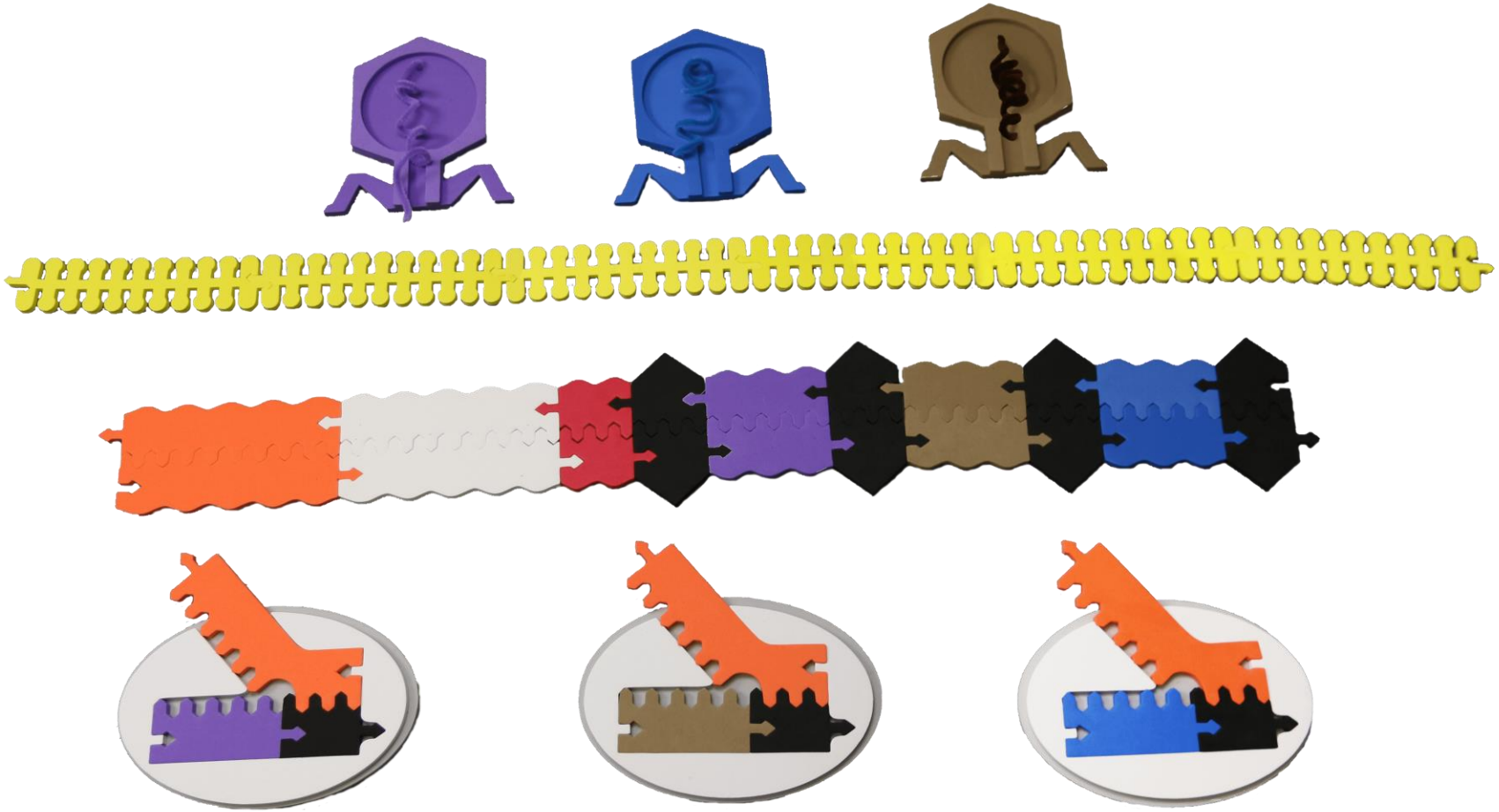
After school activities

New Teacher Mentor
SMART team advisor
Wrestling coach
Fishing club advisor

Workshop Learning Goals

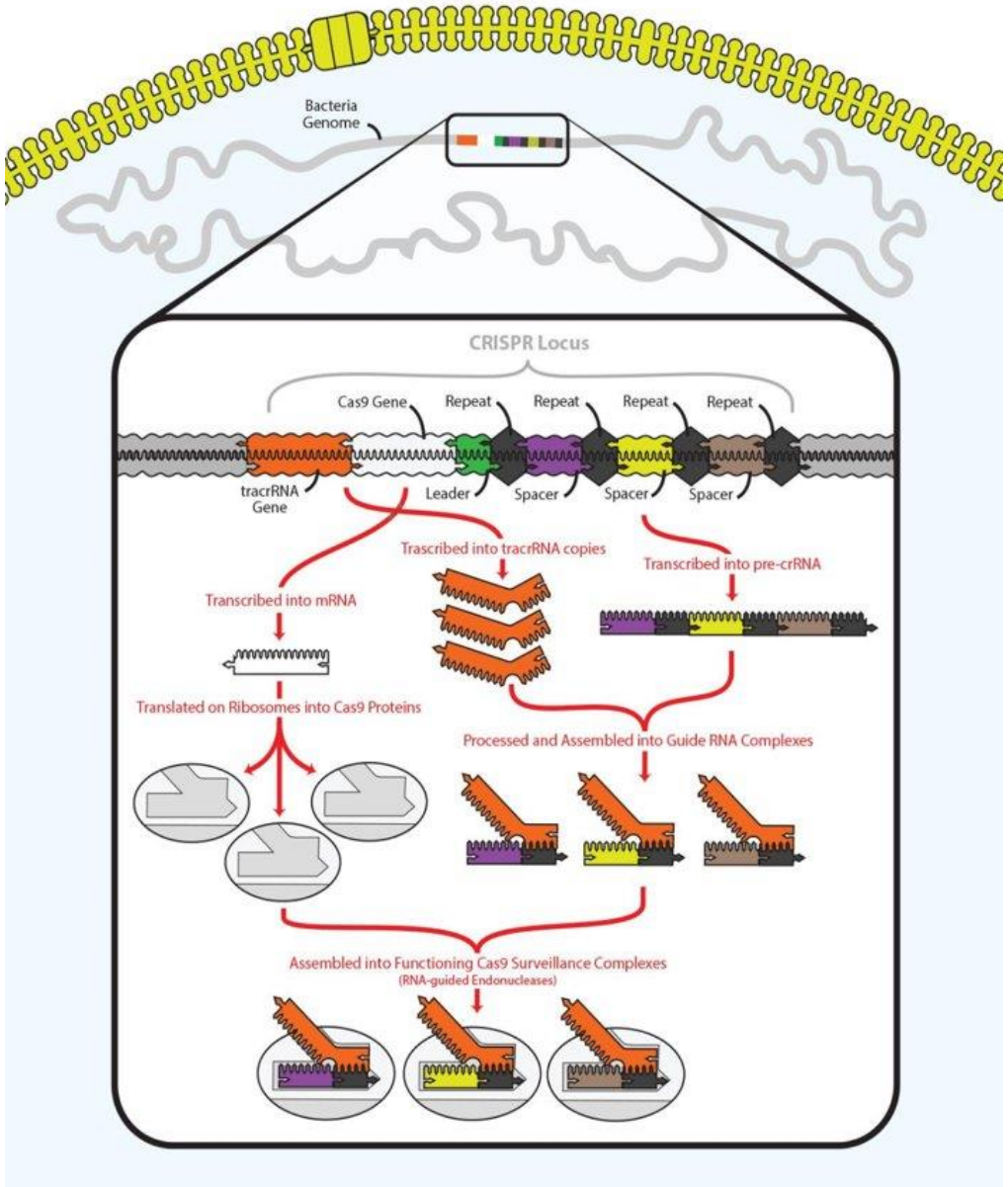
- 1) Understand how CRISPR Cas-9 defends a bacterial cell from viral infection
- 2) Model this acquired immune system in bacteria
- 3) Determine how it can be used in biotechnology
- 4) Understand how viruses use CRISPR like systems
- 5) Have fun!

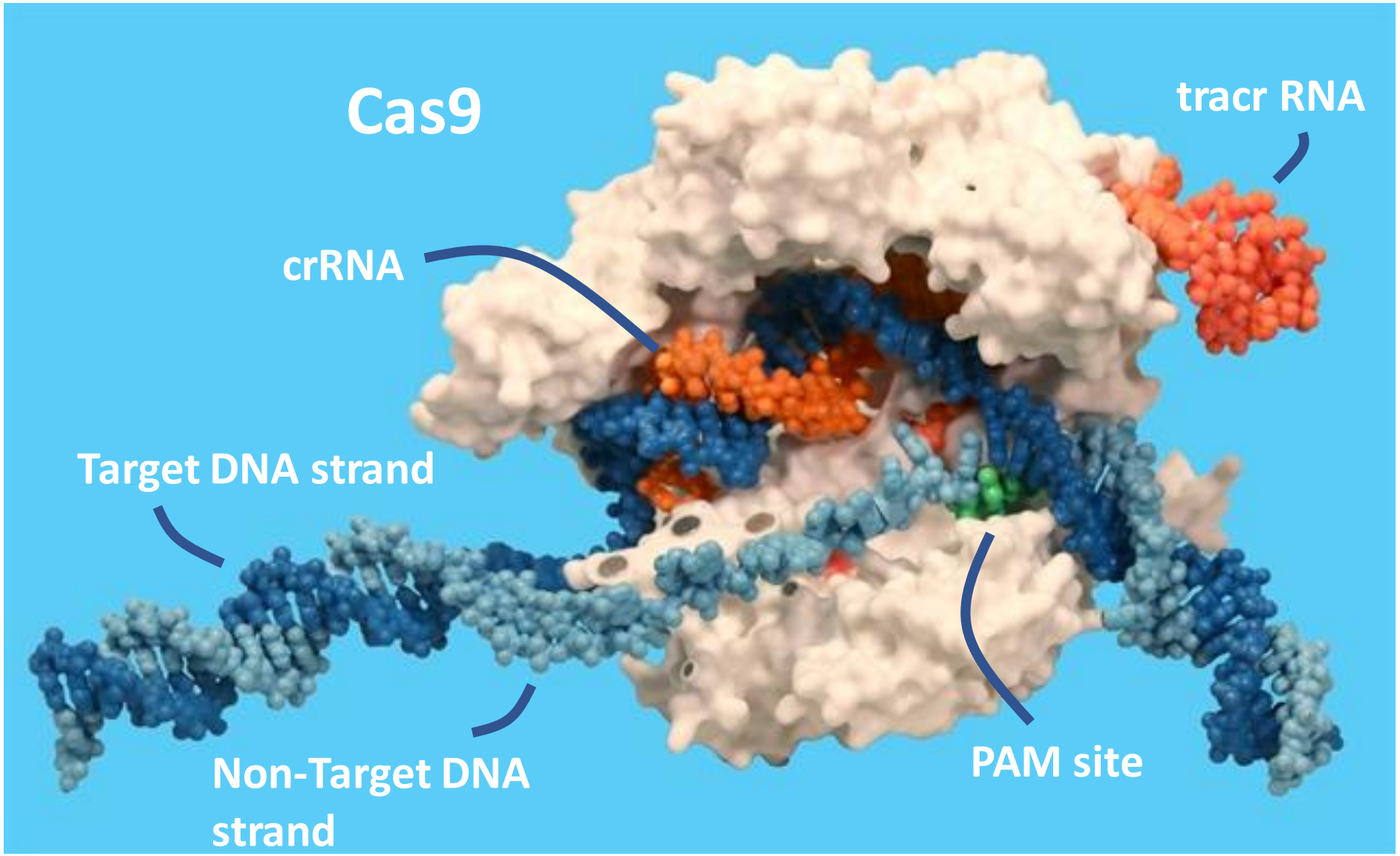
What is CRISPR?



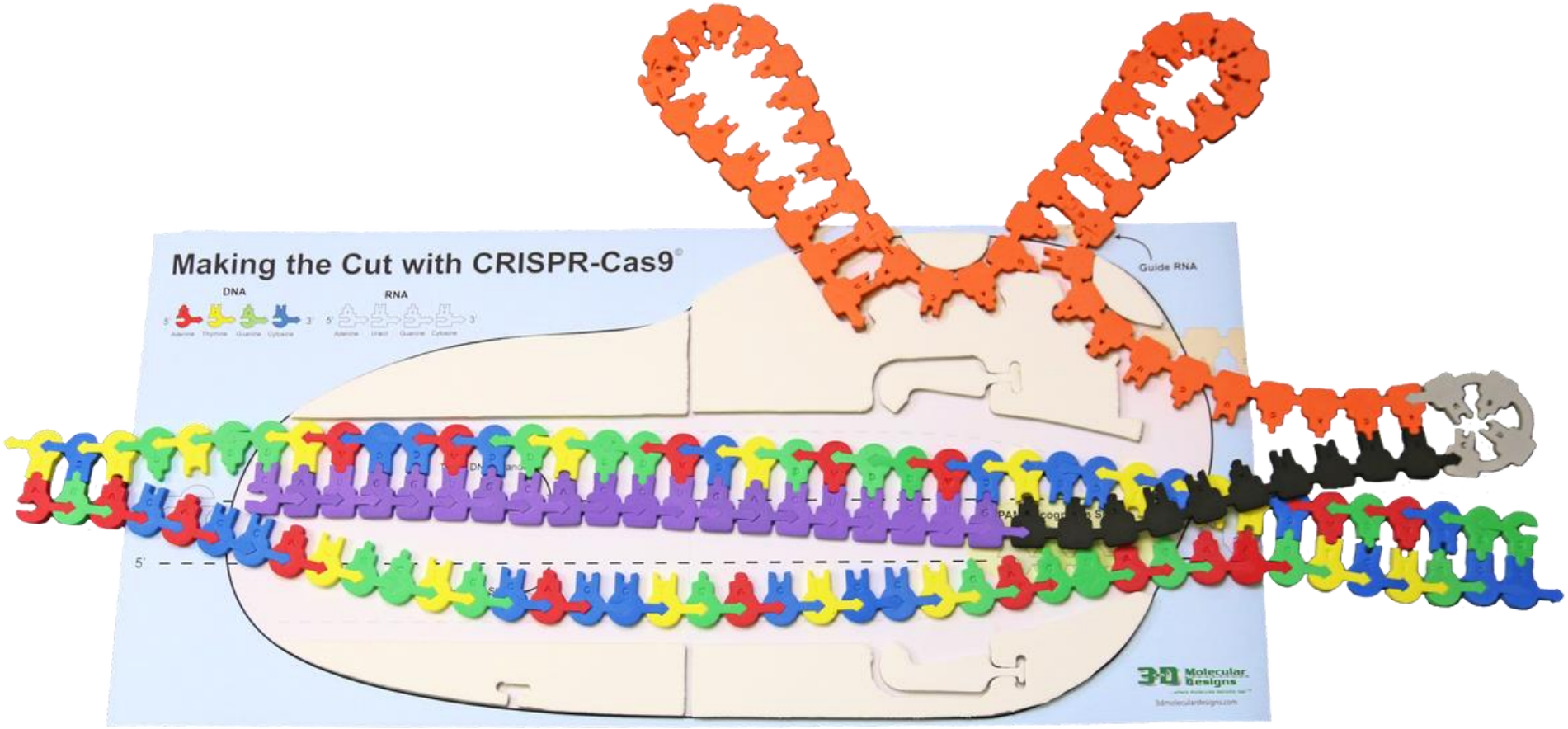
2. Expression of CRISPR Locus Genes

Building Cas9 Surveillance Complexes

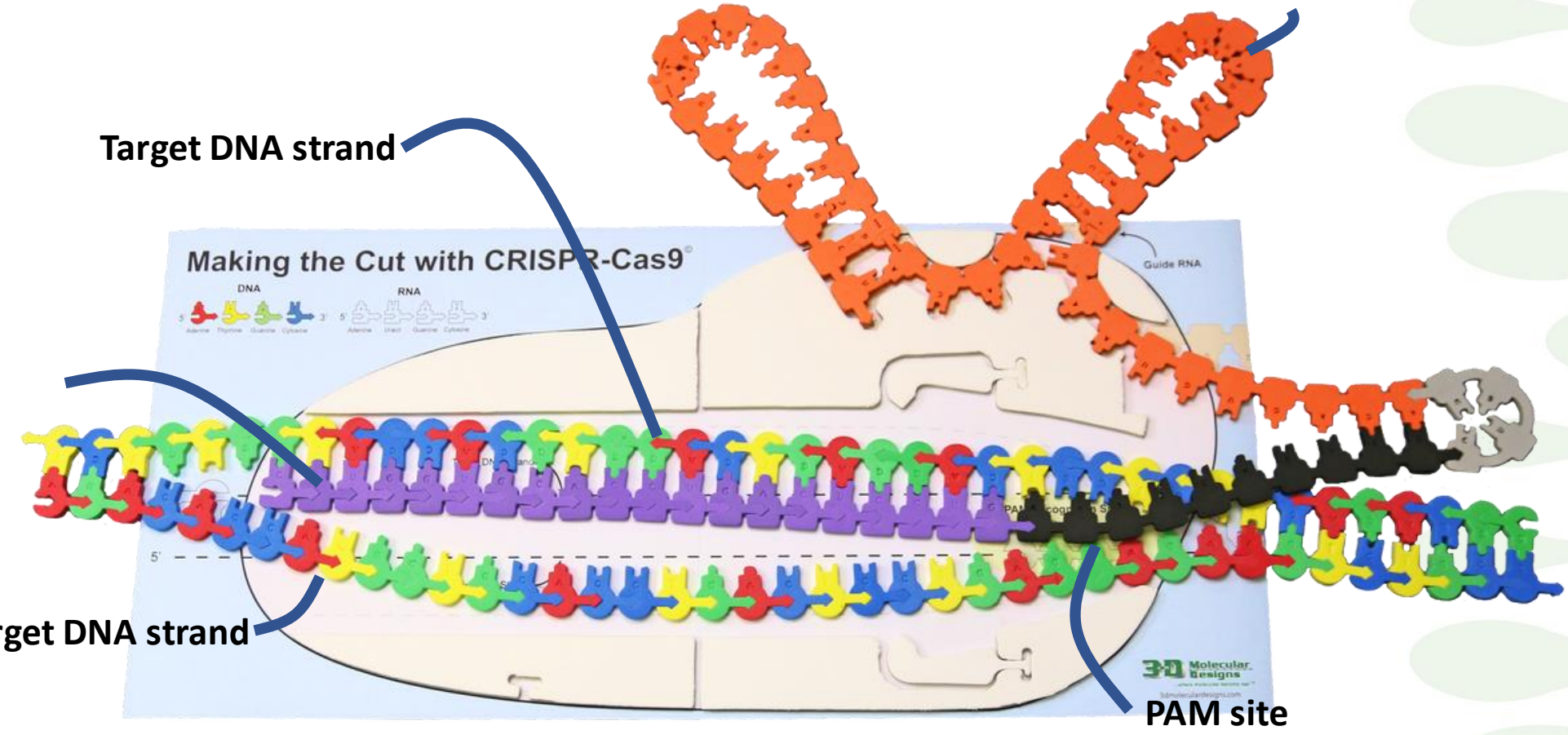



Cas9
tracr RNA
crRNA
Target DNA strand
Non-Target DNA strand
PAM site

Making the Cut with CRISPR Cas-9



Model transference

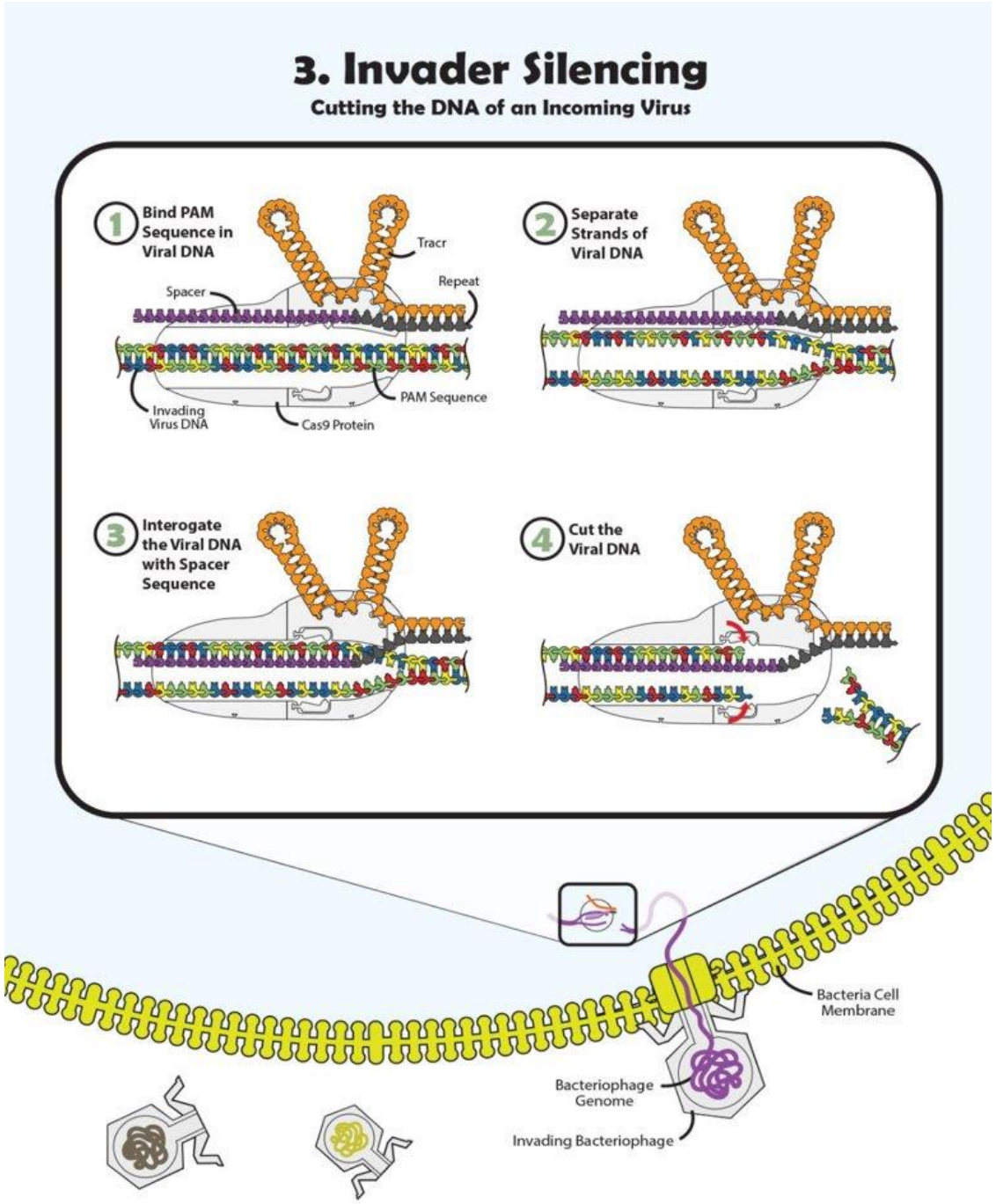


Protospacer Adjacent Motif (PAM)

- The PAM sequence for Cas-9 is NGG, where N represents any base.
- How often would we expect to see this sequence mathematically?
- G = ¼
- G = ¼
- NGG = 1/16
- So… statistically, Cas-9 would interrogate DNA every 16 base pairs

Let's Model!

- What happens inside a bacterial cell when it gets infected by a virus-
- 1) Cas-9 complex finds free floating DNA in the cytoplasm of the cell
- 2) Cas-9 interrogates DNA after identifying a PAM sequence
- 3) If DNA matches the crRNA, endonucleases activity makes a double stranded break


3. Invader Silencing
Cutting the DNA of an Incoming Virus
1 Bind PAM Sequence in Viral DNA
Tracr
Repeat
Spacer
PAM Sequence
Invading Virus DNA
Cas9 Protein
2 Separate Strands of Viral DNA
3 Interogate the Viral DNA with Spacer Sequence
4 Cut the Viral DNA
Bacteria Cell Membrane
Bacteriophage Genome
Invading Bacteriophage

STEP 1: Construct your viral DNA sequence.

Using the multi-colored foam deoxyribonucleotides (DNA), construct a model of a double-stranded viral DNA with the following nucleotide sequence:

STEP 2: Construct your crRNA (CRISPR RNA).

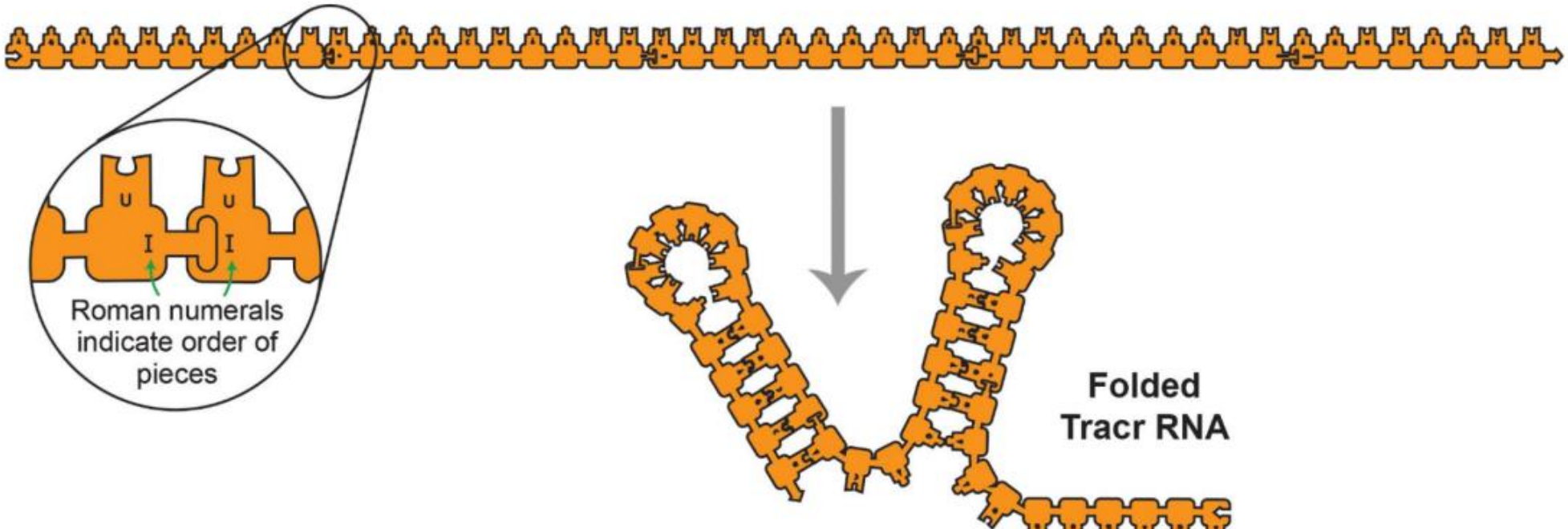
crRNA is composed of a spacer sequence and a portion of the repeat RNA. Using the purple-colored ribonucleotides (RNA), construct a model of the first 20 nucleotides of the crRNA with the following spacer sequence (note that this is complementary to a segment of the viral DNA):

Then extend the 3’ end of your spacer RNA sequence by adding the black RNA segment. This black sequence represents a segment of the CRISPR repeat sequence. This black repeat sequence, along with the purple spacer sequence represents the crRNA. crRNA is transcribed from the CRISPR array, and contains viral sequences at its 5’ end, and repeat sequences at its 3’ end.

STEP 3: Construct a model of tracrRNA.

tracrRNA is transcribed from the tracr gene. But it is ***NOT*** a messenger RNA. It is ***NOT*** translated into protein. Instead, it functions as a structural RNA. It folds up into a complex 3D shape, much like a protein. The tracrRNA is then bound in a very specific way by the Cas9 protein. (You will see in the next step that the 5' end of the tracrRNA forms complementary base pairs with the 3' end of the crRNA.)

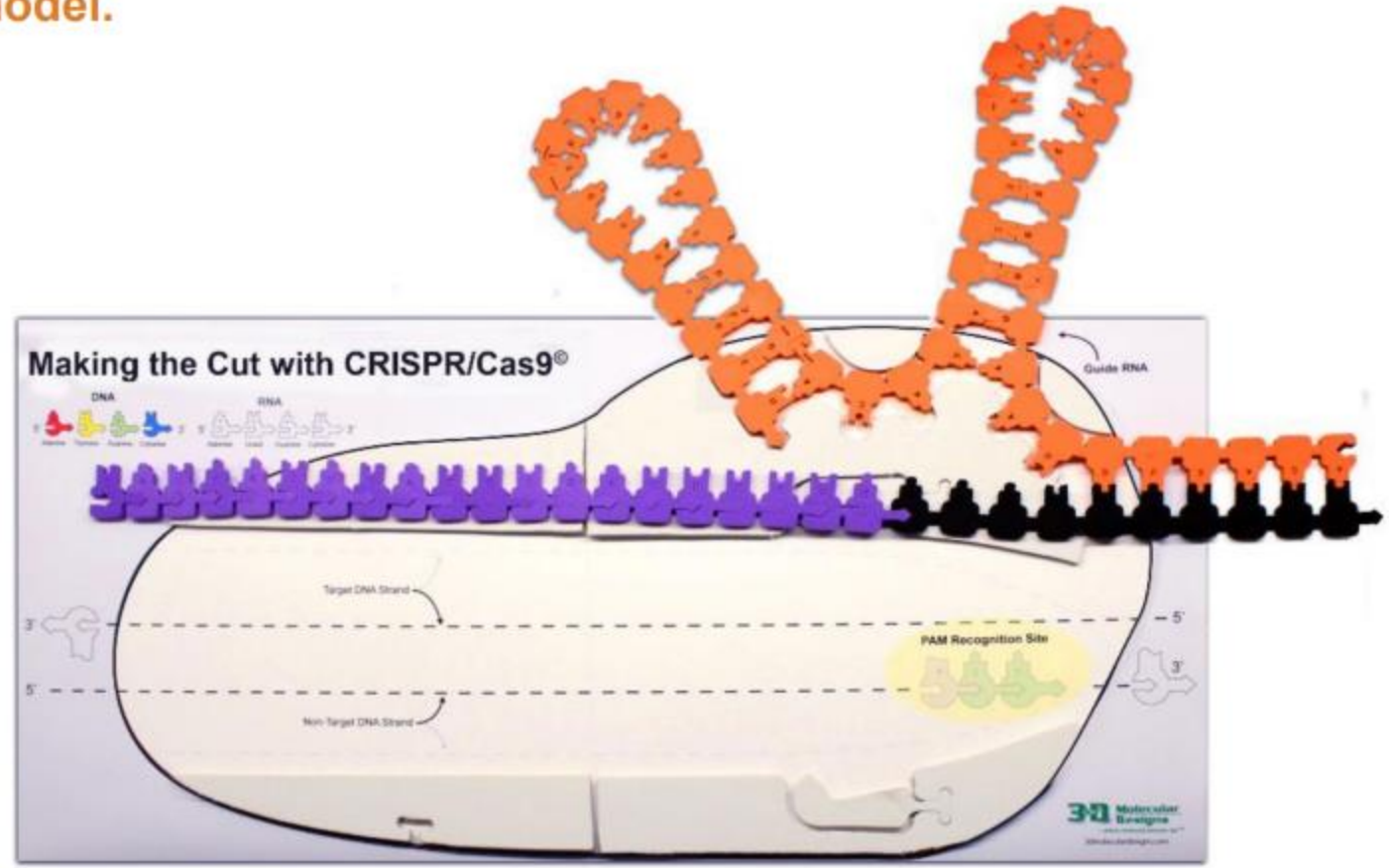
Join each of the five orange-colored RNA fragments together in the correct order, by matching the numbers at the end of each segment. Once you have made one long continuous sequence of tracr RNA, examine the sequence (starting at the 3'end) to find the complementary base pairs that results in the two "hairpins" shown below. This is simply a two-dimensional model of the very complicated 3D shape of the actual tracr RNA.



STEP 4: Construct the dual guide RNA and add it to the Cas9 protein model.

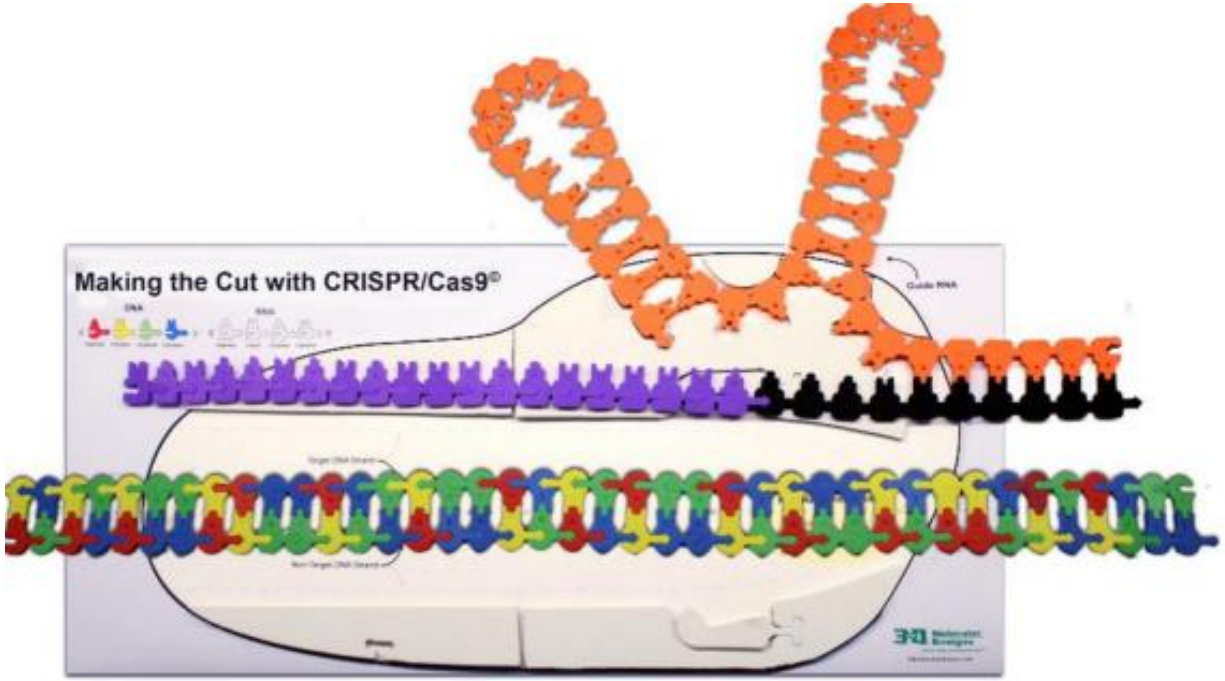
Dual guide RNA is made up of two RNAs – the tracrRNA and the crRNA. The 5'end of the tracrRNA is complementary to the 3' end of the crRNA. Add this dual guide RNA to the Cas9 protein as shown in the photo to the right. (Start by positioning the 3' end of the tracr RNA into the slot in the Cas9 model.)

Once you have added the guide RNA to Cas9, the purple viral RNA sequence is now ready to interrogate the target strand of double-stranded viral DNA. If the guide RNA spacer matches the viral DNA, Cas9 will then cut both strands of viral DNA.

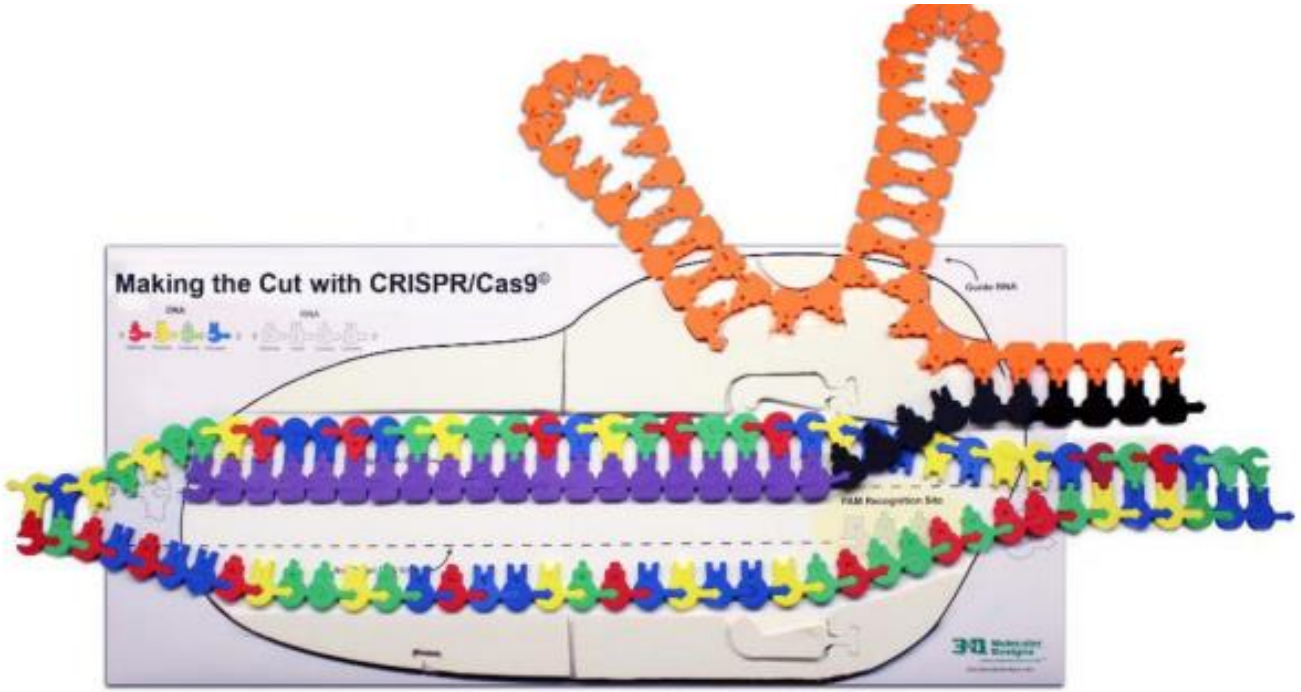


STEP 5: **Add the double-stranded viral DNA sequence to the Cas9 protein.**

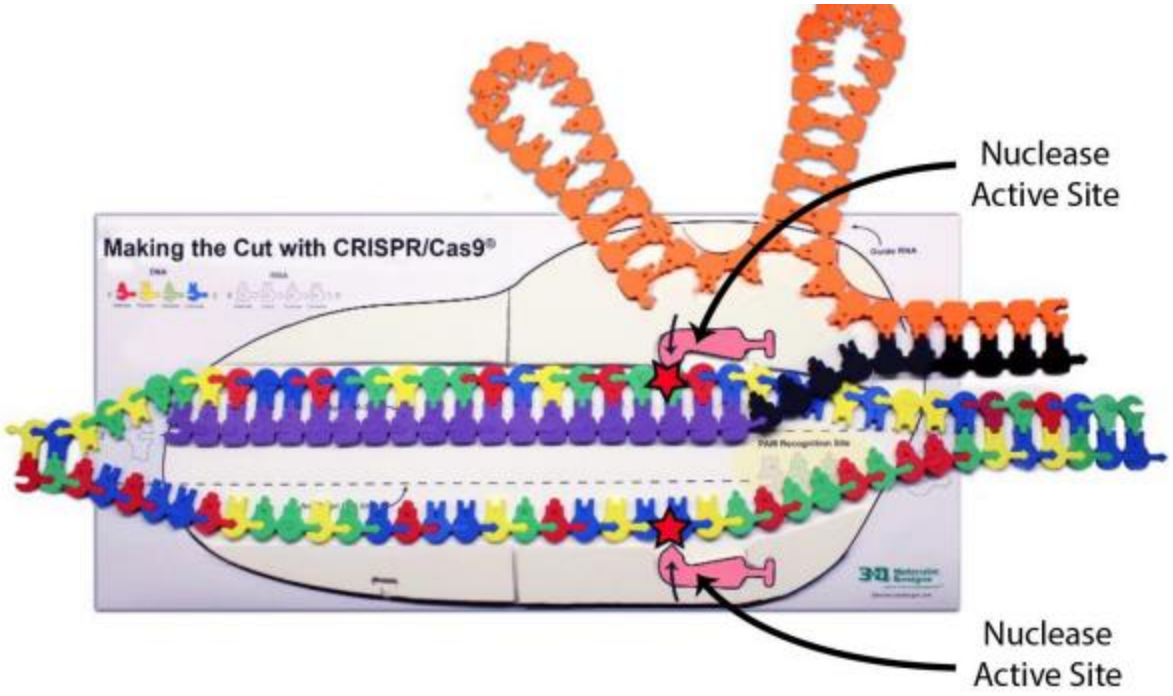
The Cas9/guide RNA complex binds double-stranded DNA, but only at a PAM site. A PAM site (Protospacer Adjacent Motif) is the sequence 5'-NGG-3', where N is any nucleotide. Therefore, take the double-stranded DNA sequence that you assembled and add it to the schematic Cas9 model with a PAM site positioned over the site indicated on the Cas9 model. (Note that there are several other PAM sequences found in this double-stranded DNA sequence).



Once the DNA is bound by Cas9 at the indicated PAM site, separate the two DNA strands, so that the purple viral RNA sequence can interrogate the top/target DNA strand.



If the viral RNA sequence is complementary to the top/target DNA strand, the two separate nuclease active sites of Cas9 are activated, and both strands of DNA are cut. The cut occurs 3 bases upstream of the PAM site.



As a result, Cas9 has created a double-stranded, blunt end cut at a specific sequence in the viral DNA. ***Congratulations***, you have just destroyed the viral genome, and prevented the infection of the bacteria.

So what?

Restriction enzymes

Hae III is an endonuclease that digests DNA at

5’ GG/ CC 3’
3’ CC/ GG 5’

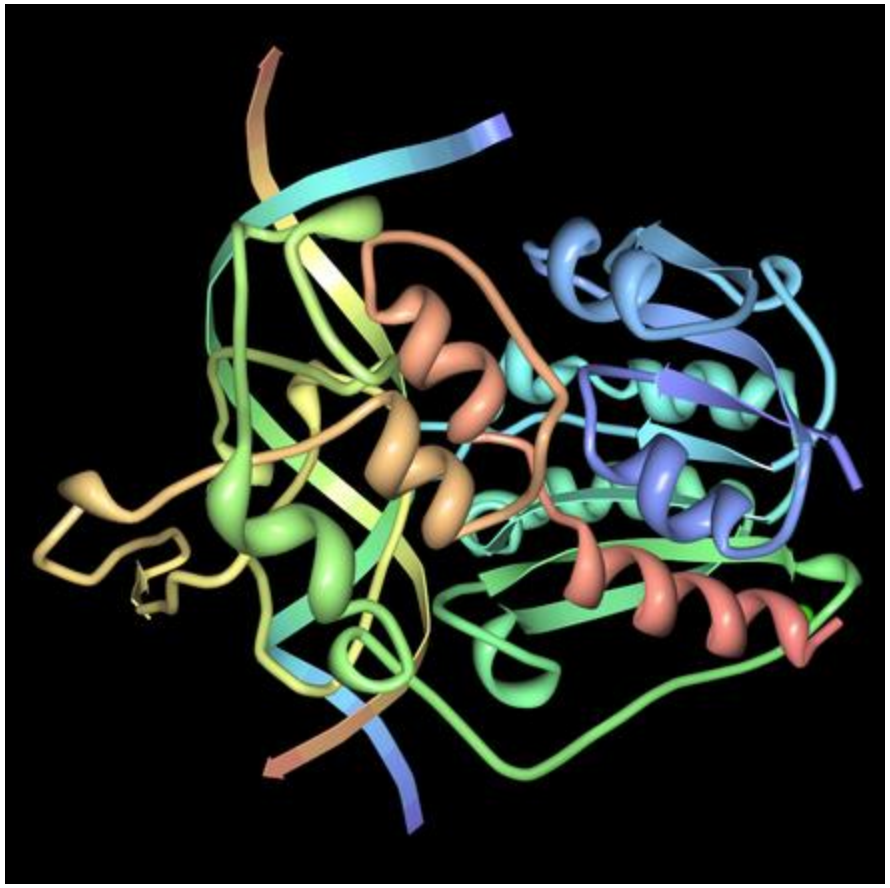
We could use this for a gene knock out – turning a gene off.

… but …

¼ x ¼ x ¼ x ¼ = 1/256

Statistically… 3.2 billion base pairs would be cut into 12.5 million fragments!

This would be ideal if your goal was to turn off ALL OF THE GENES!



Edit the genome?

Cas-9 gives us a highly specific DNA targeting system – All we need to do is write the RNA guide sequence!

What makes this so powerful? I can target a ~20 bp sequence out of 3.2 billion!

For perspective:
One line of genetic code – Times New Roman – 12 font
AATCCCGTGGGGTTTCTTTTTATCTAAATCTTTATTACTTTATTTTCGGGGTATTTATTCTGTTTTAACTTGCACATTTATCTGAATTTGCGGGGGGGATCTTCTAT

2,502 bp on an 8.5" x 11" sheet of paper, one side = 5004 on both sides

The human genome would be a book 639,489 pages long.
500 sheets of high-quality paper is 2.5" (unbound)
(639,489 pages / 500 sheets) * 2.5" = " / 12" = 266.5 feet thick

In Football Terms…
Our epic genome book on its side would go from our goal line to the opposing teams almost 11-yard line.
(88.8 yards)

This programed machine will find the ATGCTTGGCTAAGTCCACGT sequence on p. 434,473

Guide RNA

- CRISPR systems use 17-24 RNA base pairs to interrogate the target strand after identifying the PAM sequence.

- How many base pairs would we need to find a statistically unique sequence in the human genome? (3.2 billion base pairs)

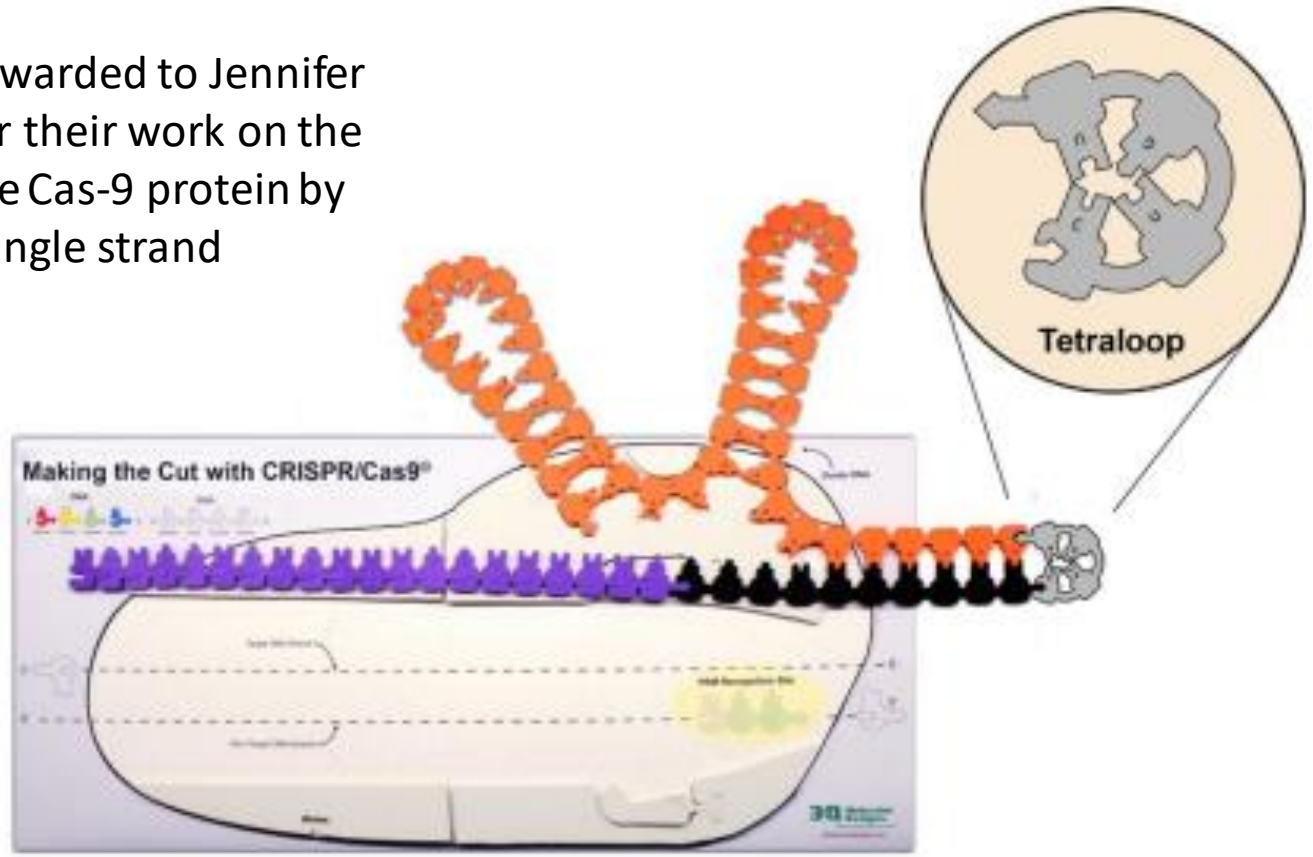
- That's ¼ to the 16th power… or 1 / 4,300,000,000

... and it still hits off target…

How is that possible?

Doudna and Charpentier

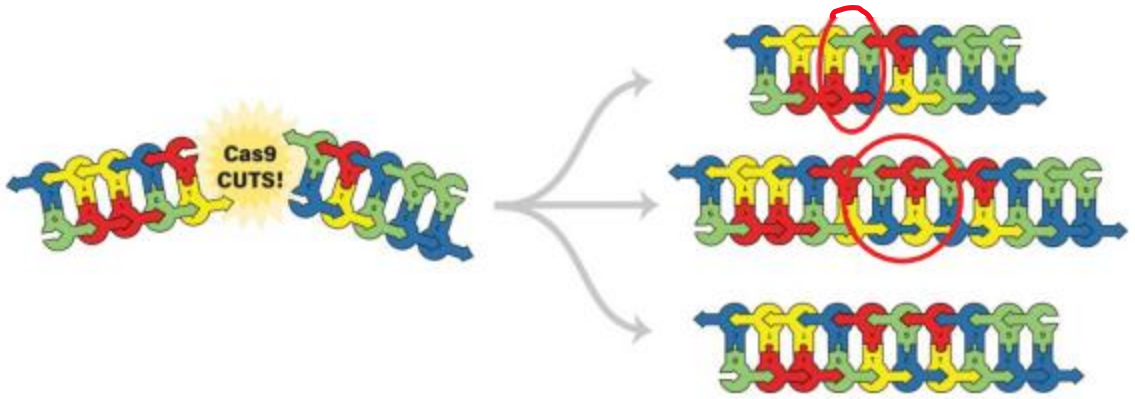
The 2020 Nobel Prize in Chemistry was awarded to Jennifer Doudna and Emmanuelle Charpentier for their work on the Tetraloop which allows for a programable Cas-9 protein by making the tracrRNA and crRNA a long single strand



DNA with a double stranded break (DSB)

- In your genome, a DSB is … well… bad. Real bad!
- Your body has 2 ways to repair a DSB

Non-Homologous End-Joining (NHEJ)



Very error prone – repair the break at all costs, even if it introduces some mistakes

*Knock-out

Homology Directed Repair (HDR)

- Broken DNA is repaired using a template-
- It’s complicated

- The short of it-

- Template DNA matches up with broken strand 3’ sticky end - enzymes make a single strand until it matches the other side – Other enzymes ligate and duplicate other strand - *see video

Base editing

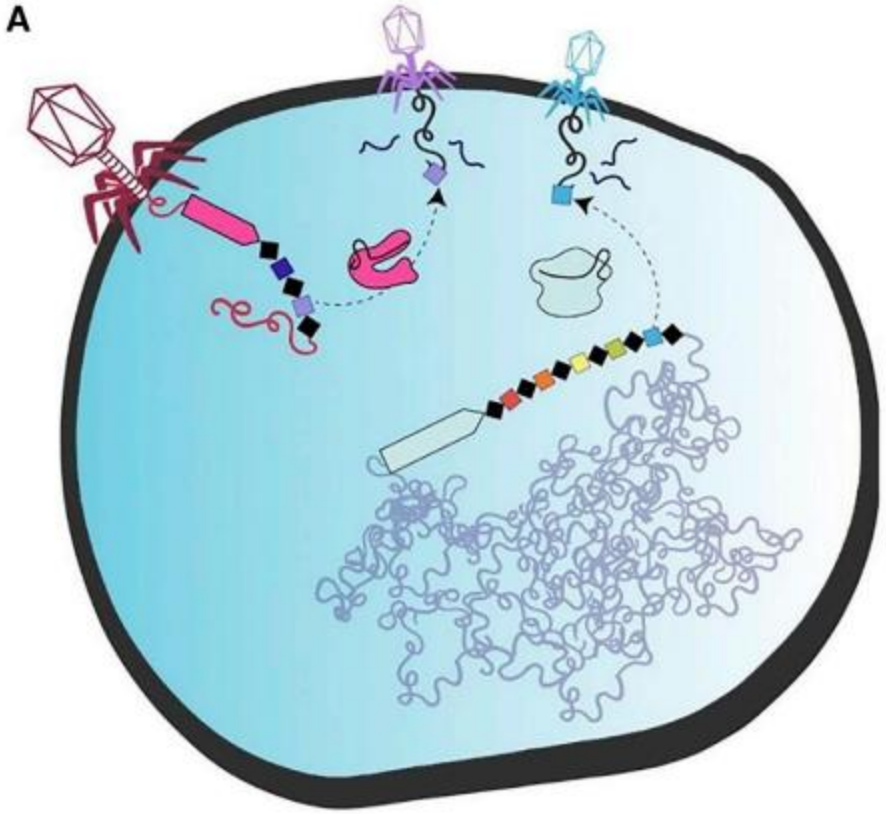
- Researchers are now using CRISPR in very clever ways

- David Liu -> Cas-9 directed base editing
- Use Cas-9 to direct base editors to an exact location
- C -> T by Cytidine deaminase tethered to Cas-9

- Nick the non-edited strand – marked for repair.

- “Fix” a single nucleotide polymorphism from C-G base pair to a T-A – accounts for ~14% of SNP pathology

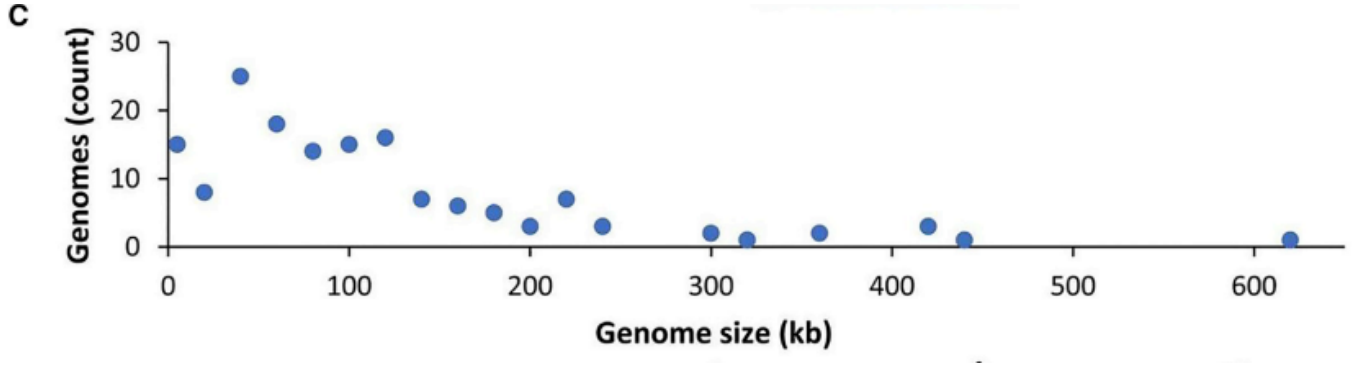
Viruses using CRISPR like proteins?



A study conducted on viral genomes indicate that many viruses have different CRISPR like systems.

These CRISPR like systems are used to gain a monopoly on cellular resources by preventing other viruses from infecting the cell

Researchers are currently investigating these smaller CRISPR like proteins for potentially editing “difficult to edit“ cells



Additional resources

A Programable dual-RNA-guided DNA Endonuclease in adaptive bacterial immunity
Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012 Aug 17;337(6096):816-21. doi: 10.1126/science.1225829. Epub 2012 Jun 28. PMID: 22745249; PMCID: PMC6286148.

Diverse virus-encoded CRISPR-Cas systems include streamlined genome editors (cell.com)
Al-Shayeb, B., Skopintsev, P., Soczek, K. M., Stahl, E. C., Li, Z., Groover, E., . . . Doudna, J. A. (2022). Diverse virus-encoded CRISPR-CAS systems include streamlined genome editors. *Cell, 185*(24). doi:10.1016/j.cell.2022.10.020

HDR – Video

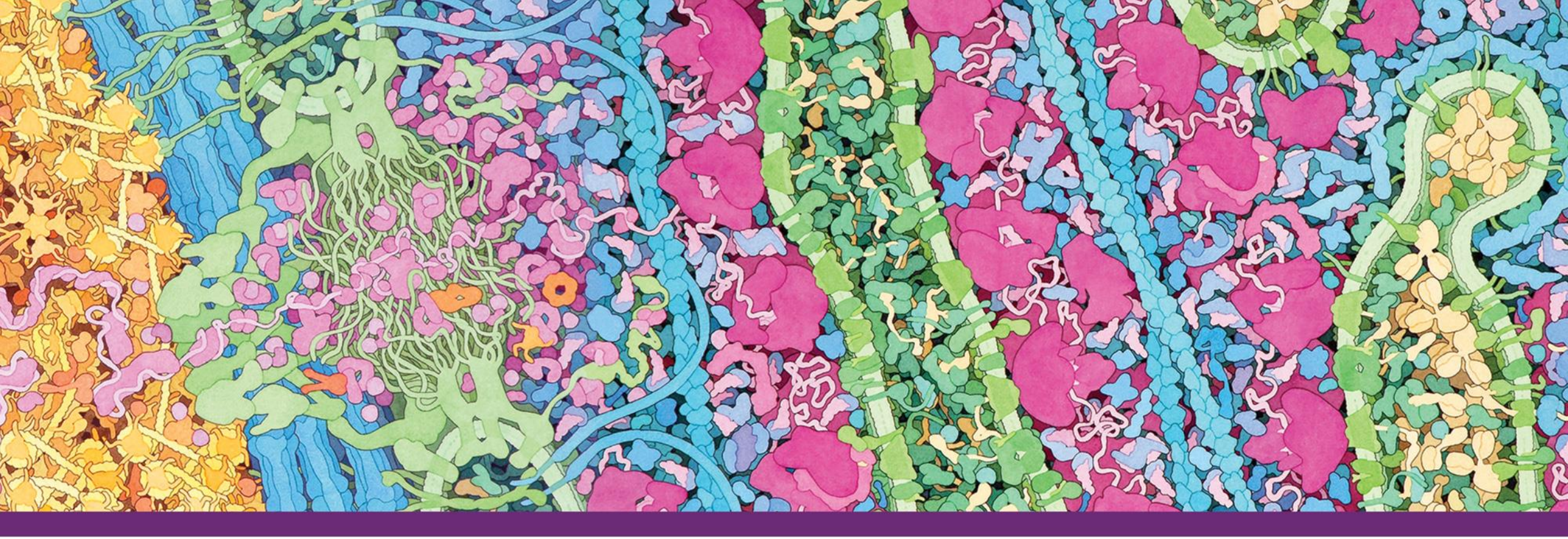
NHEJ video

David Liu – Can we cure genetic diseases by rewriting DNA? (Ted Talk)

Workshop NGSS Connections

SEPs	CCCs	DCIs
Asking Questions	**Patterns**	**HS-LS1-1**
Developing and Using Models	**Cause and Effect**	**HS-LS1-2**
Constructing Explanations	**Scale, Proportion, and Quantity**	**LS-1A**
	Stability and Change	**LS-1C**
		LS-1D

Scan me!



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