Genome Editing II

CRISPR Cas9: A Powerful New Tool for Editing the Human Genome

CRISPR Innovations: Clever and Cleverer

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Genome Editing Webinar
Wednesday, March 10, 2021

Recording will be posted 1 or 2 days after the webinar.

Tim Herman, PhD, MSOE Center for BioMolecular Modeling & 3D Molecular Designs

Session Resources

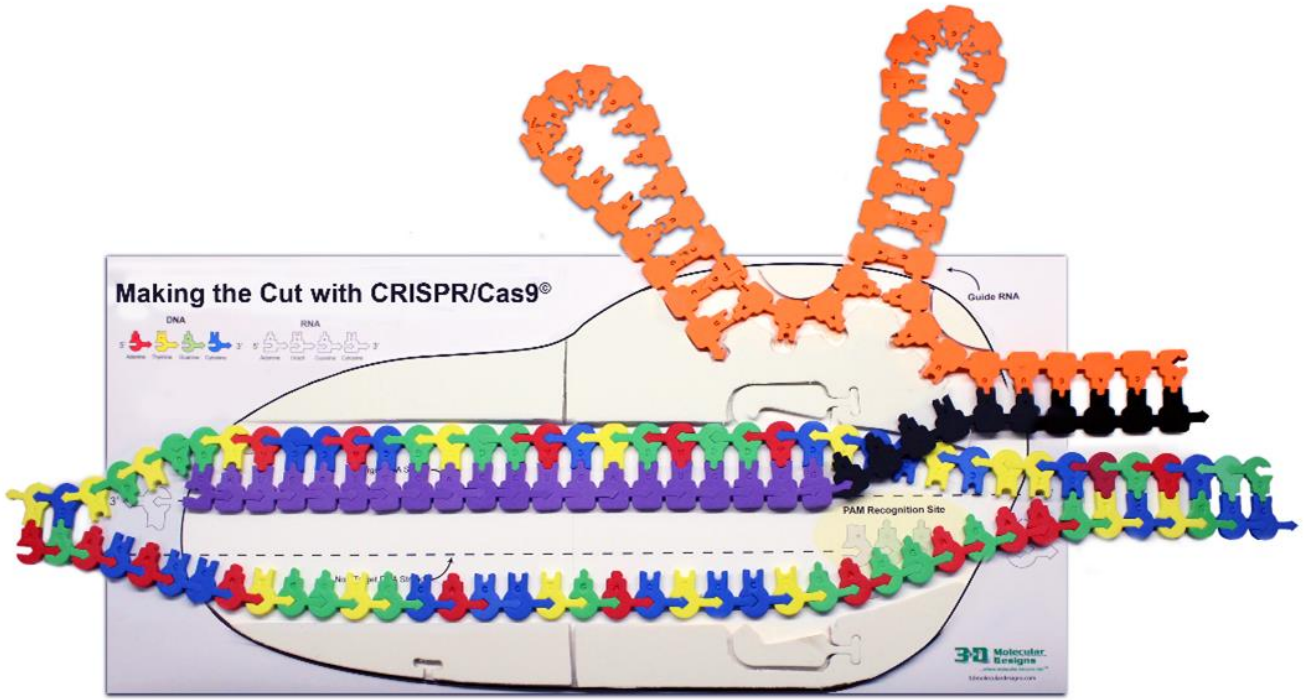
- CRISPR Adaptive Immunity System 3-Step Guiding Graphics
- "CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes" article from 2007 *Science*
- "Microbial Warfare Against Viruses" article from 2018 *Science*

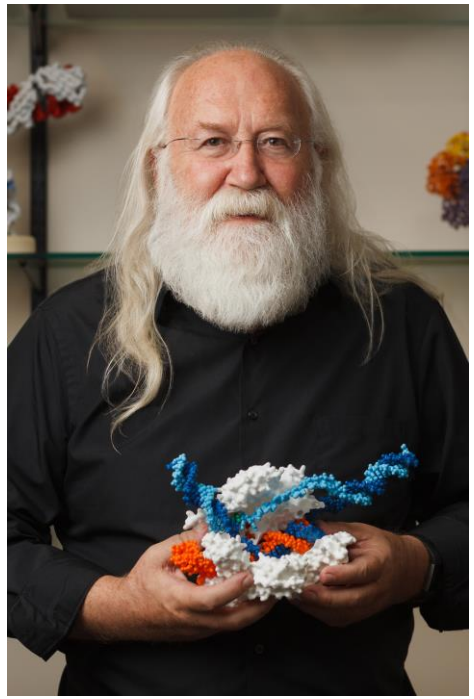
3D Molecular Designs' Products

- Teacher Resources

Center for BioMolecular Modeling Resources

- Model Lending Library


Making the Cut with CRISPR/Cas9©
DNA
RNA
Guide RNA
PAM Recognition Site
Molecular Designs



How does Cas9 work? & What makes Cas9 special?

CRISPR … an Adaptive Immunity System in bacteria

This CRISPR system deploys an RNA-guided Cas9 endonuclease

How does Cas9 protect bacteria from bacteriophages?

first, restriction enzymes,…. and then, CRISPR Cas9

The CRISPR Cas9 Kit ….tracr RNA; crRNA; PAM sites

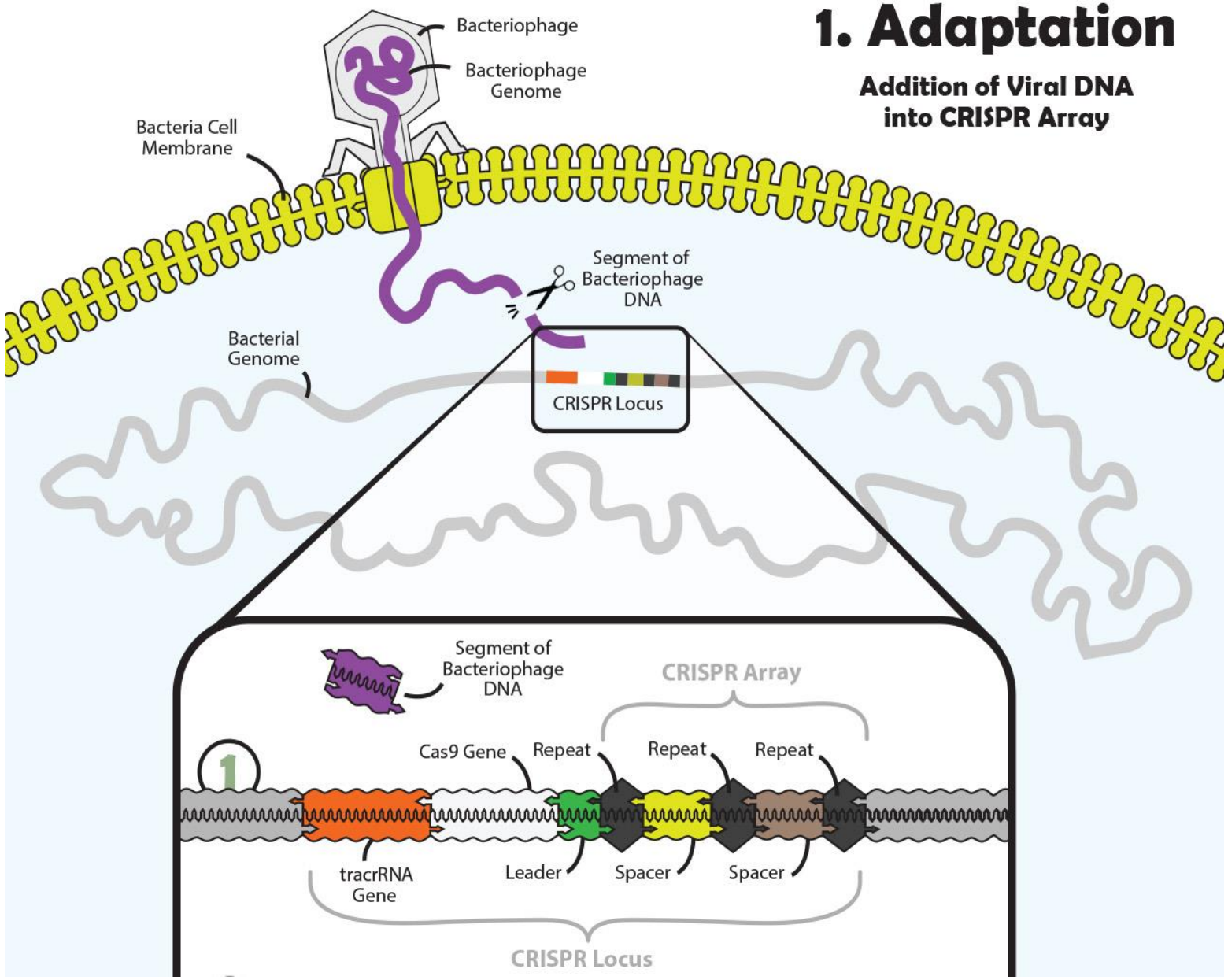
What happens after the cut?

NHEJ…..Non-Homologous End Joining

HDR…Homology Directed Repair

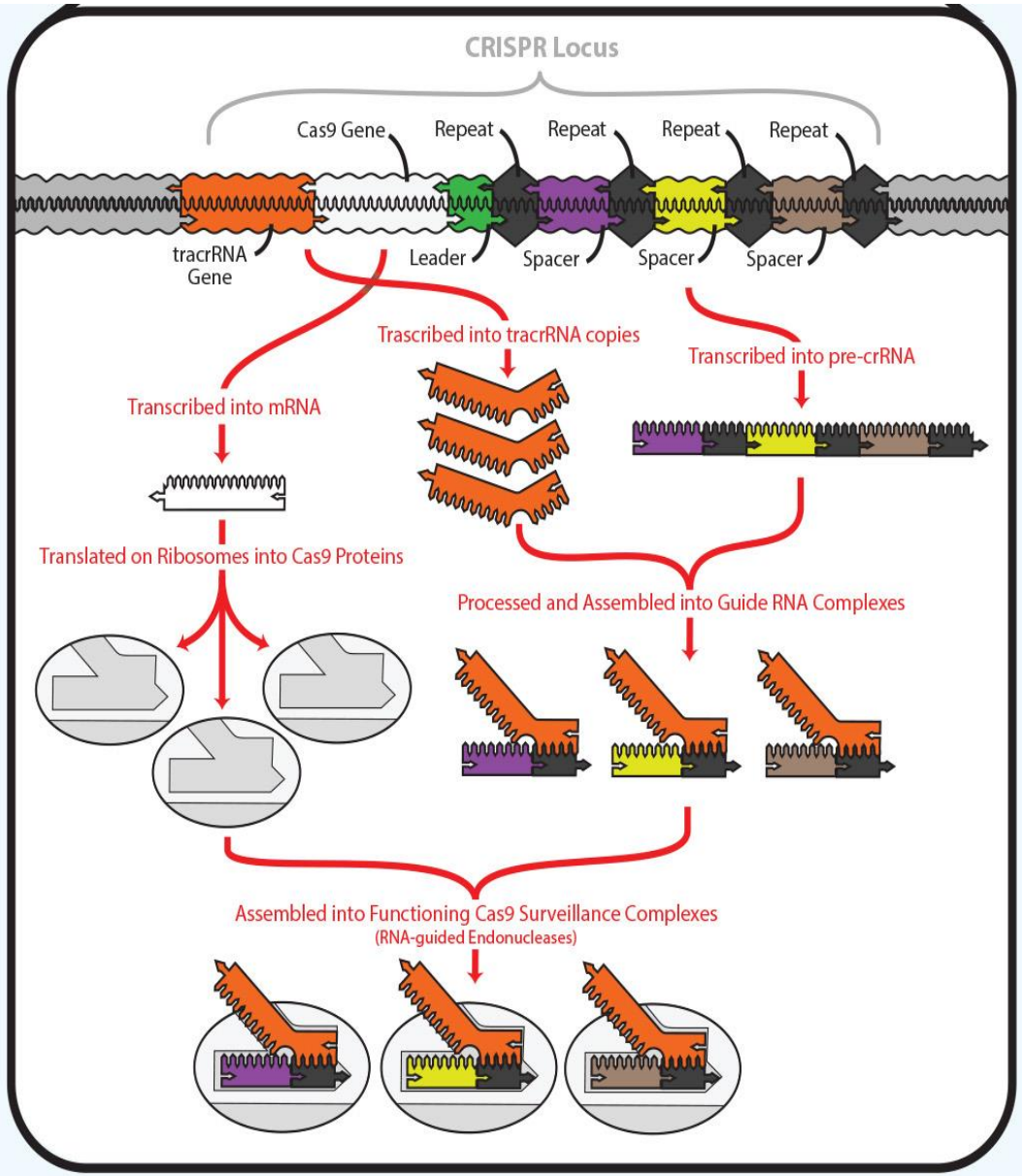
A 3D-printed model of Cas9…and a Cas9 Molecular Exploration.

Engineering Cas9 to be a better editor…. Introducing *base editing*


1. Adaptation
Addition of Viral DNA into CRISPR Array
Bacteriophage
Bacteriophage Genome
Bacteria Cell Membrane
Segment of Bacteriophage DNA
Bacterial Genome
CRISPR Locus
Segment of Bacteriophage DNA
CRISPR Array
Cas9 Gene
Repeat
Repeat
Repeat
1
tracrRNA Gene
Leader
Spacer
Spacer
CRISPR Locus

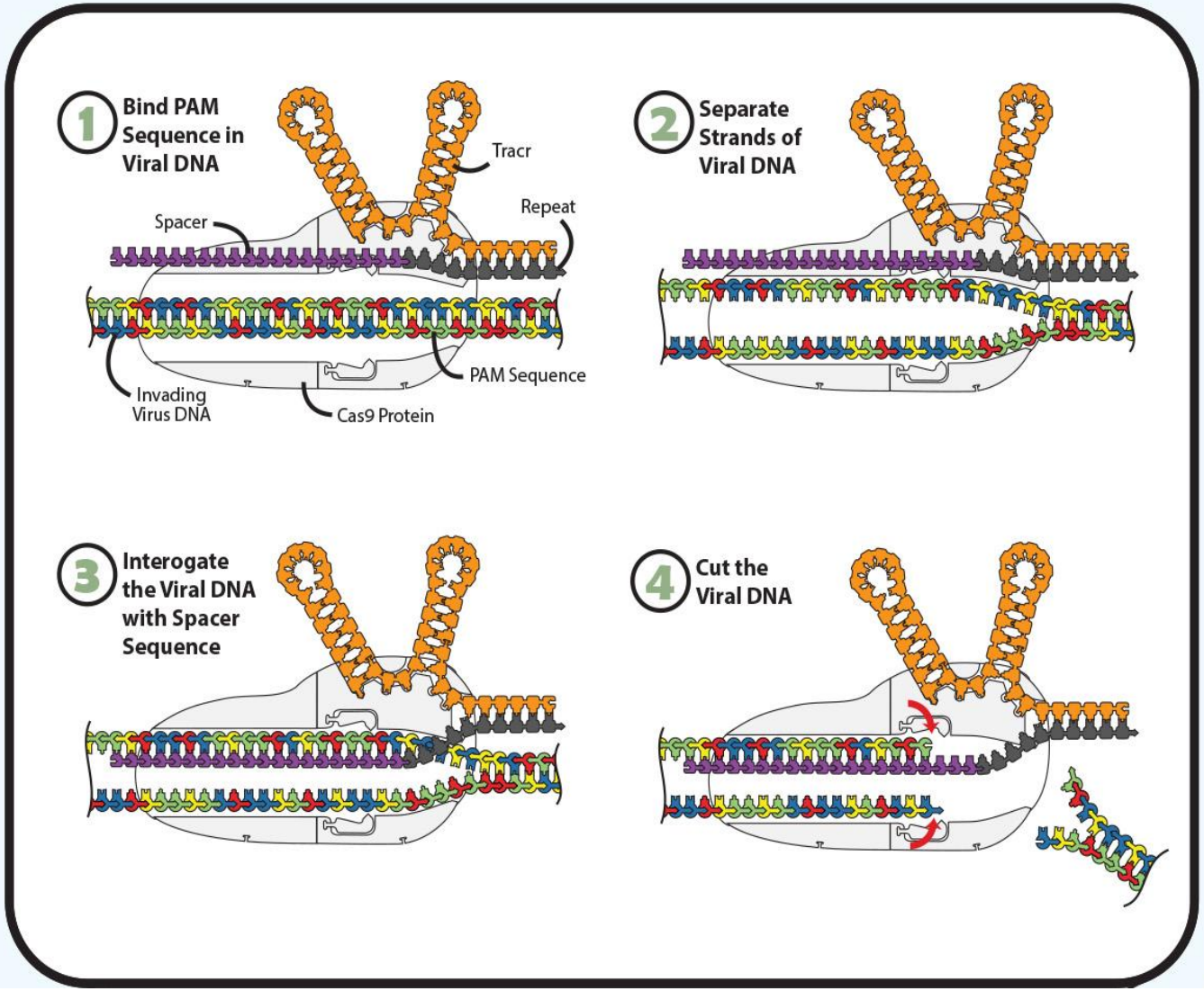
2. Expression of CRISPR Locus Genes

Building Cas9 Surveillance Complexes

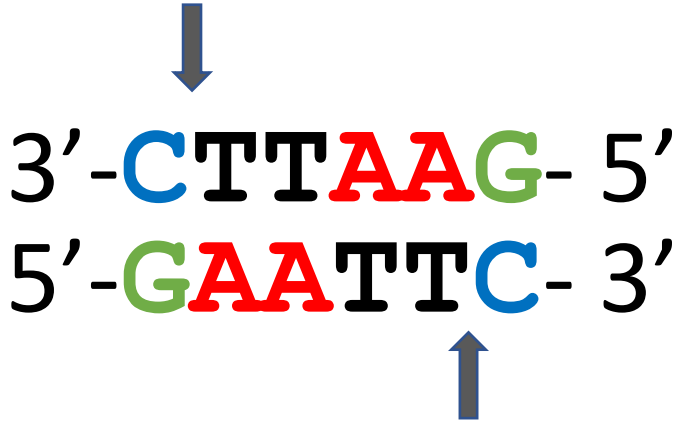


3. Invader Silencing

Cutting the DNA of an Incoming Virus



_Eco_RI… 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 *Eco*RI 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:

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

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… 16 24 36 42 ?

A statistically unique site in the human genome…

GAATTCGTCGCTAATG

16 nucleotides

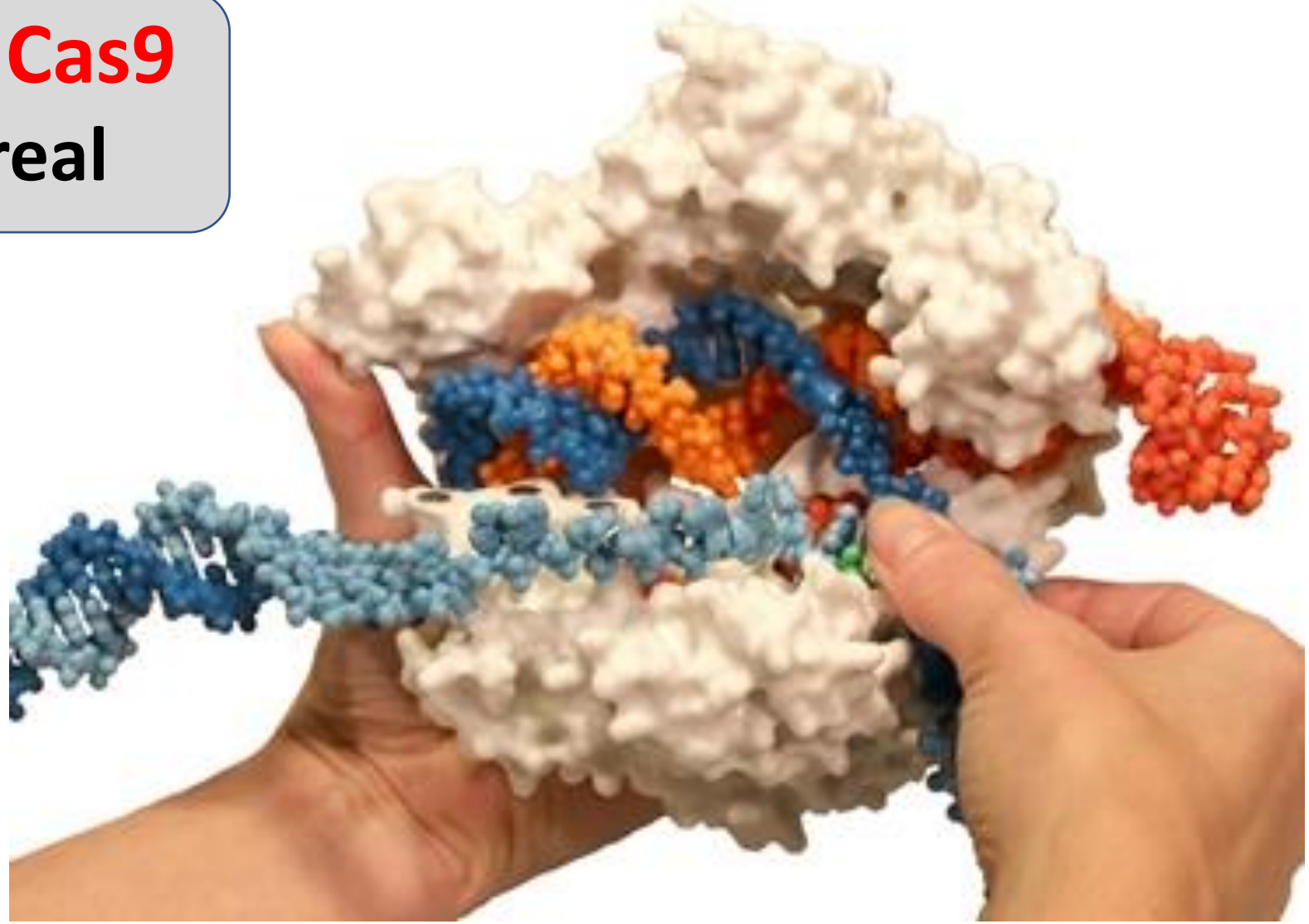
4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 x 4 = 4.3 X 10^9

…or…

4^{16} = 4.3 X 10^9

CRISPR Cas9

… for real



Making the Cut with CRISPR/Cas9

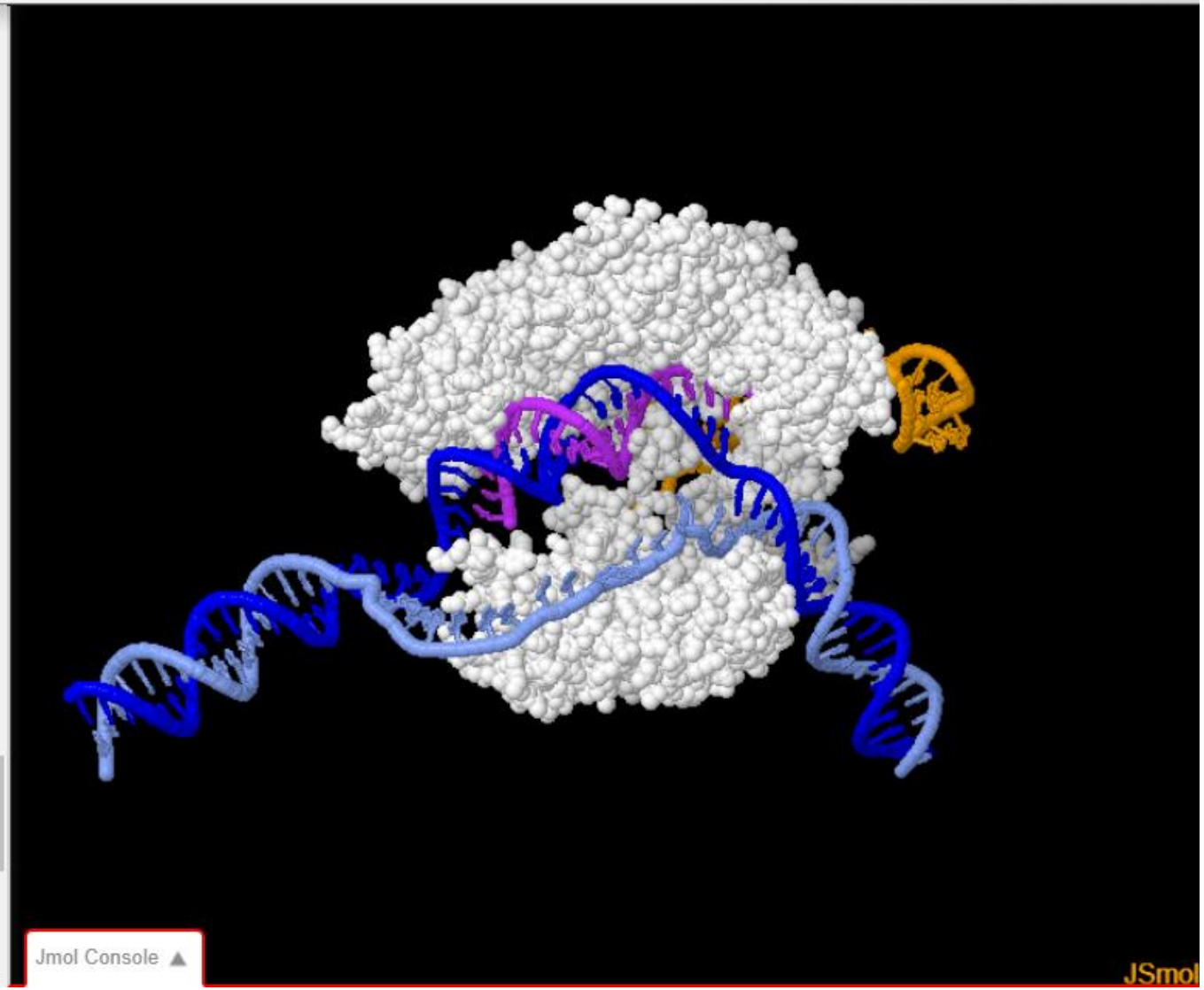
An Interactive Jmol Exploration

notice about either of these two separated strands of DNA?

One strand of the opened DNA, known as the "target strand", lines up directly with the "spacer" of the guide RNA, almost as if the spacer is trying to test if it is complementary to the DNA, and can form A-T/G-C base-pairs.

Keeping in mind where the purple "spacer" section of the guide RNA originally came from, what would it mean if they were indeed complementary?

It would mean that the DNA being scanned matches the sequence of the virus that previously infected the bacteria, and that the same virus has come attacking again! Now what does that poor bacteria do?!




A
D
B
C
G
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E
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J
I
3-D Molecular Designs
...where molecules become real™

QUESTION: ***What happens after the cut?***

```
ATTGCCAGTCAGATCAGAGGTAA ?  CTTACGGTGCATGACATTACTAGT
TAACGGTCAGTCTAGTCTCCATT    GAATGCCACGTACTGTAATGATCT
```

ANSWER: ***The cell tries to repair the damage!***

Two Options

or

Non-Homologous End Joining

(Error-prone)

↓

Gene Knock-Out

Homology Directed Repair

(Requires a homologous donor DNA)

↓

Gene Replacement

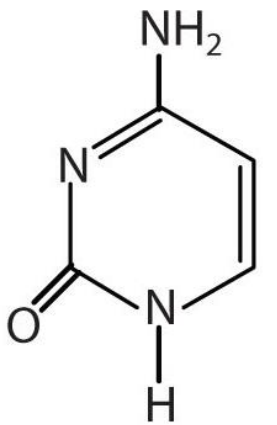
Neither outcome warrants the use of the word "editing".

David Liu (Harvard), *minding his metaphors*

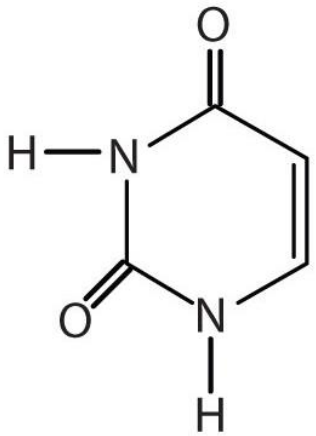
Base Editing

Almost No cutting necessary !

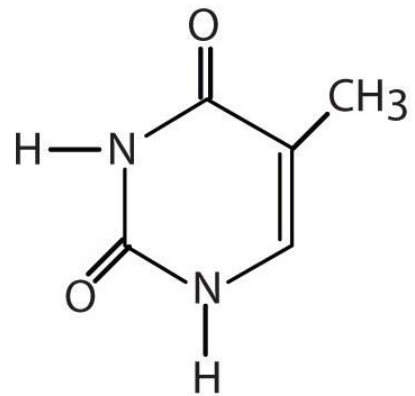
C → (Cytidine deaminase) → U → (DNA replication) → T



Cytosine (C)



Uracil (U)



Thymine (T)

LETTER

doi:10.1038/nature17946

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

Alexis C. Komor[1,2], Yongjoo B. Kim[1,2], Michael S. Packer[1,2], John A. Zuris[1,2] & David R. Liu[1,2]

Current genome-editing technologies introduce double-stranded (ds) DNA breaks at a target locus as the first step to gene correction[1,2]. Although most genetic diseases arise from point mutations, current approaches to point mutation correction are inefficient and typically induce an abundance of random insertions and deletions (indels) at the target locus resulting from the cellular response to dsDNA breaks[1,2]. Here we report the development of 'base editing', a new approach to genome editing that enables the direct, irreversible conversion of one target DNA base into another in a programmable manner, without requiring dsDNA backbone cleavage or a donor template. We engineered fusions of CRISPR/

the efficiency of gene correction relative to HDR without introducing an excess of random indels. Catalytically dead Cas9 (dCas9), which contains Asp10Ala and His840Ala mutations that inactivate its nuclease activity, retains its ability to bind DNA in a guide RNA-programmed manner, but does not cleave the DNA backbone[7]. In principle, conjugation of dCas9 with an enzymatic or chemical catalyst that mediates the direct conversion of one base to another could enable RNA-programmed DNA base editing.

The deamination of cytosine (C) is catalysed by cytidine deaminases[8] and results in uracil (U), which has the base-pairing properties of thymine (T). Most known cytidine deaminases operate on

technology feature

Base editing a CRISPR way

A way to gene edit without double-stranded DNA breaks is now entering labs.

Vivien M

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invoking
follow do
a donor template. Proponents say base
editing is an easier way to deliver editing

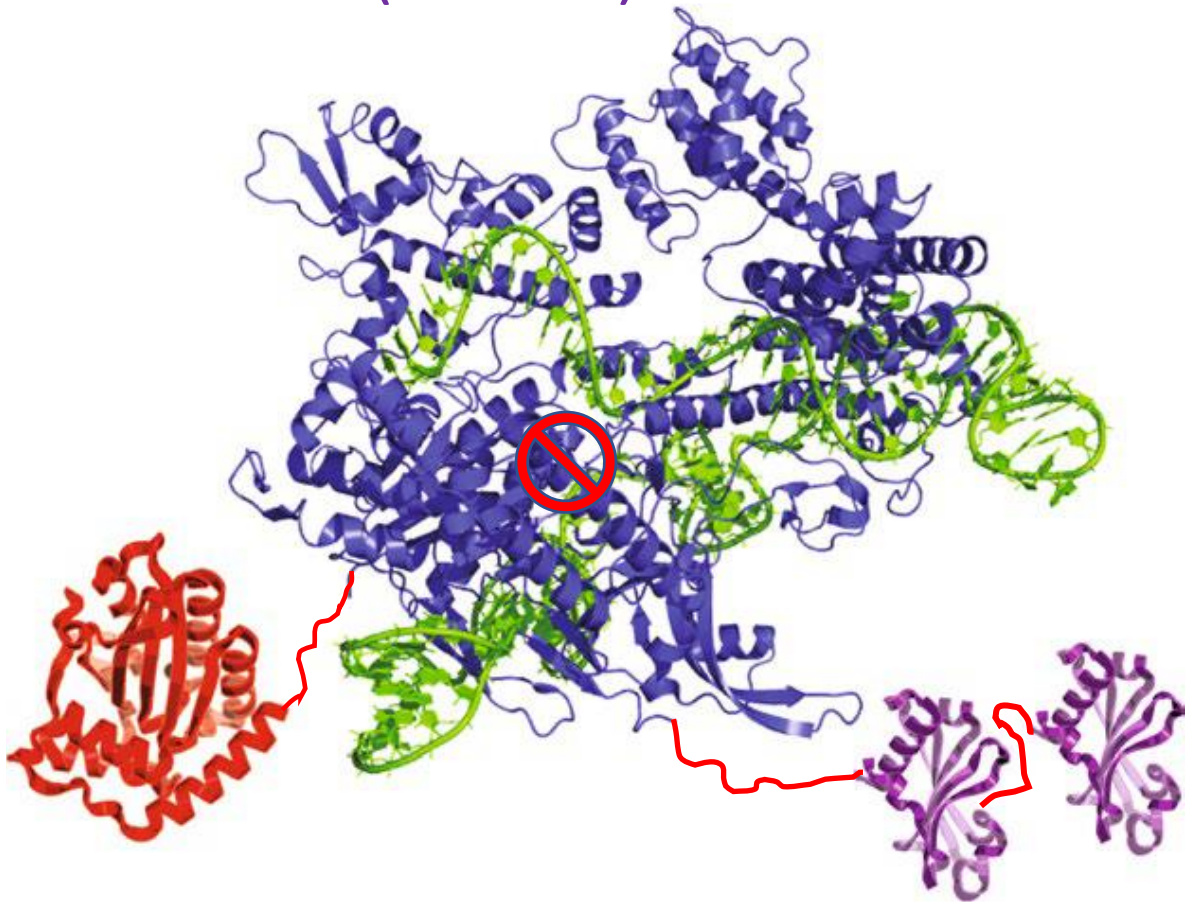
This nucleotide conversion happens without double-stranded cutting by fully active CRISPR–Cas9 nuclease, without invoking DNA repair mechanisms that follow double-stranded breaks or using a donor template. Proponents say base editing is an easier way to deliver editing

Published online: 1 October 2018
https://doi.org/10.1038/s41592-018-0146-4

BE3

...the 3rd generation Base Editor

Cas9 (nickase)



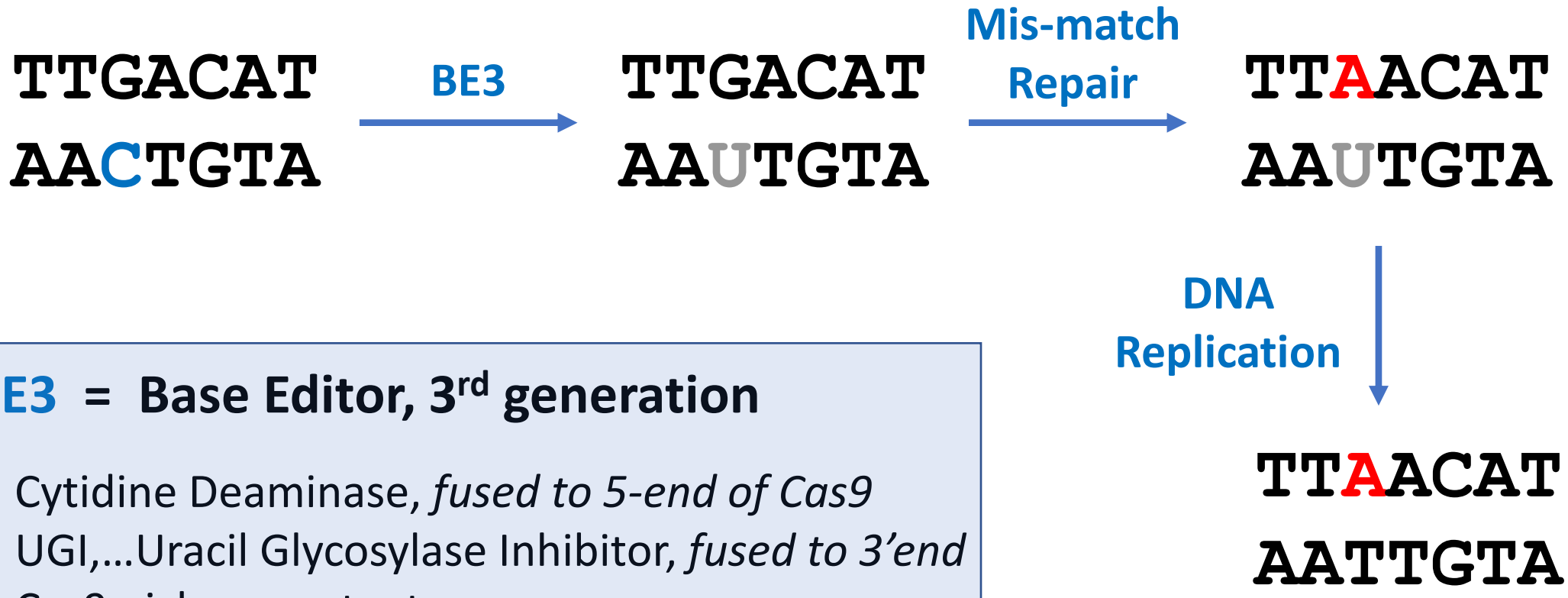
Cytidine Deaminase

Uracil Glycosylase Inhibitor... X2

David Liu

Is science cool, or what?

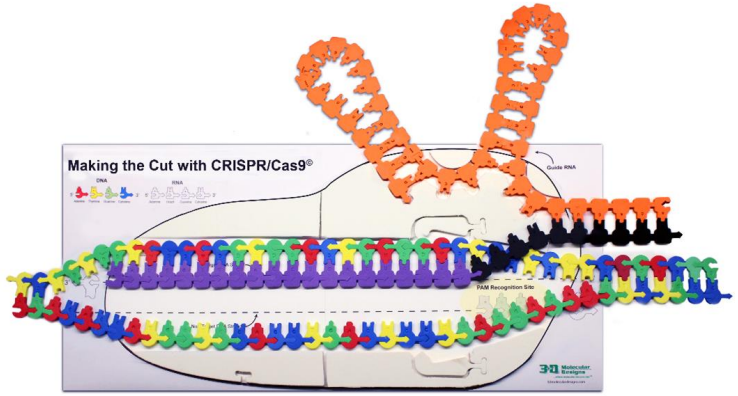
A Third Generation Base Editor…

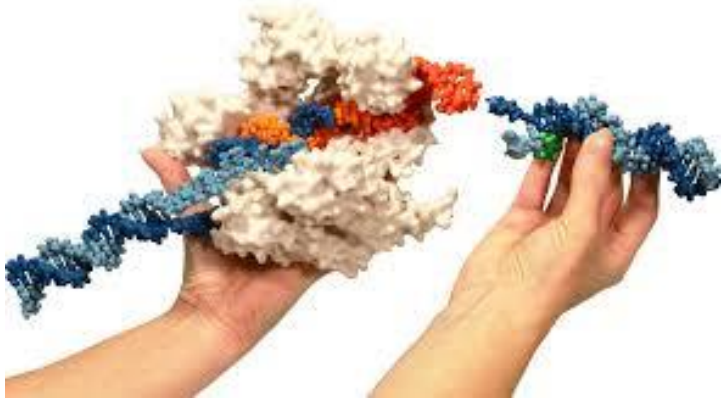


BE3 = Base Editor, 3rd generation

- Cytidine Deaminase, *fused to 5-end of Cas9*
- UGI,…Uracil Glycosylase Inhibitor, *fused to 3'end*
- Cas9 nickase mutant

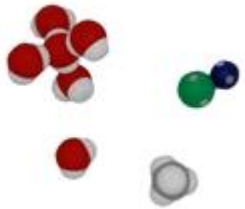
Is science cool, or what?





Thank you for attending!

Invite your colleagues to register!



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