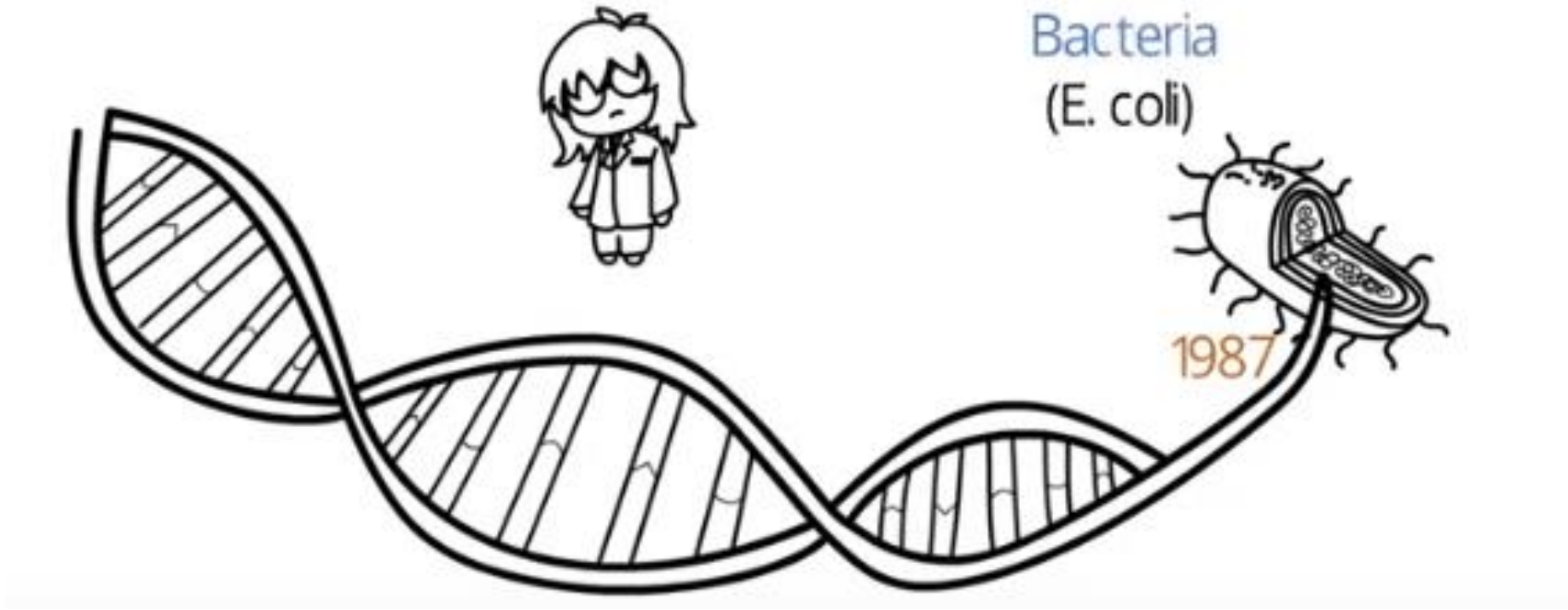
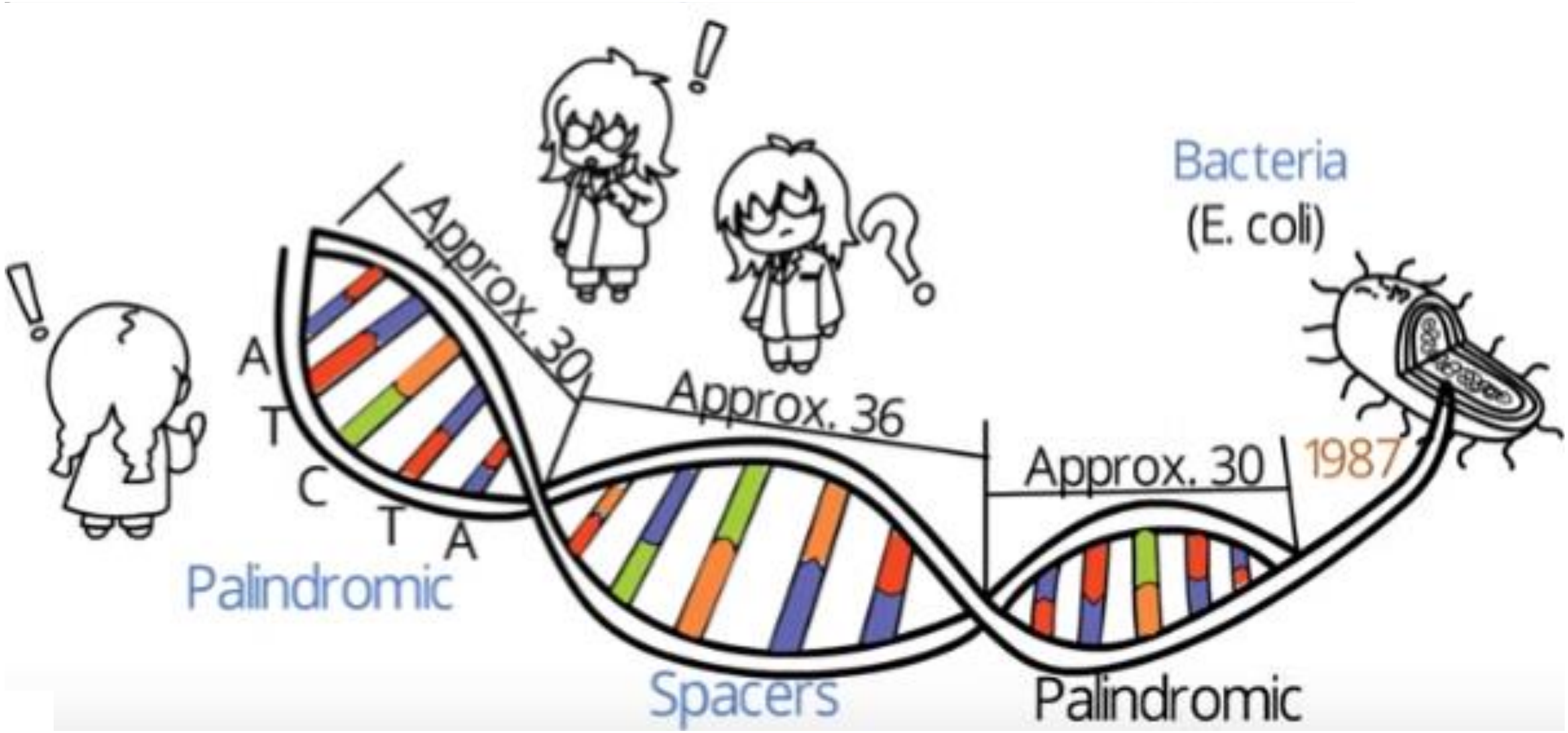
Clustered Regularly Interspersed Palindromic Repeats

Who discovered CRISPR?




Bacteria
(E. coli)
Approx. 30
Approx. 36
Approx. 30
1987
A
T
C
T
A
Palindromic
Spacers
Palindromic

- is a family of DNA sequences found within the genomes of prokaryotic organisms such as bacteria and archaea.
- These sequences are derived from DNA fragments of bacteriophages that have previously infected the prokaryote and are used to detect and destroy DNA from similar phages during subsequent infections.
- These sequences play a key role in the antiviral defense system of prokaryotes.
- **Cas9** (or "CRISPR-associated protein 9") is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence.
- Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR-Cas9 that can be used to edit genes within organisms.
- This editing process has a wide variety of applications including basic biological research, development of biotechnology products, and treatment of diseases.
- RNA harboring the spacer sequence helps Cas (CRISPR-associated) proteins recognize and cut foreign pathogenic DNA.
- CRISPR are found in approximately 50% of sequenced bacterial genomes and nearly 90% of sequenced archaea.

JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429–5433
0021-9193/87/125429-05$02.00/0

Vol. 169, No. 12

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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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.

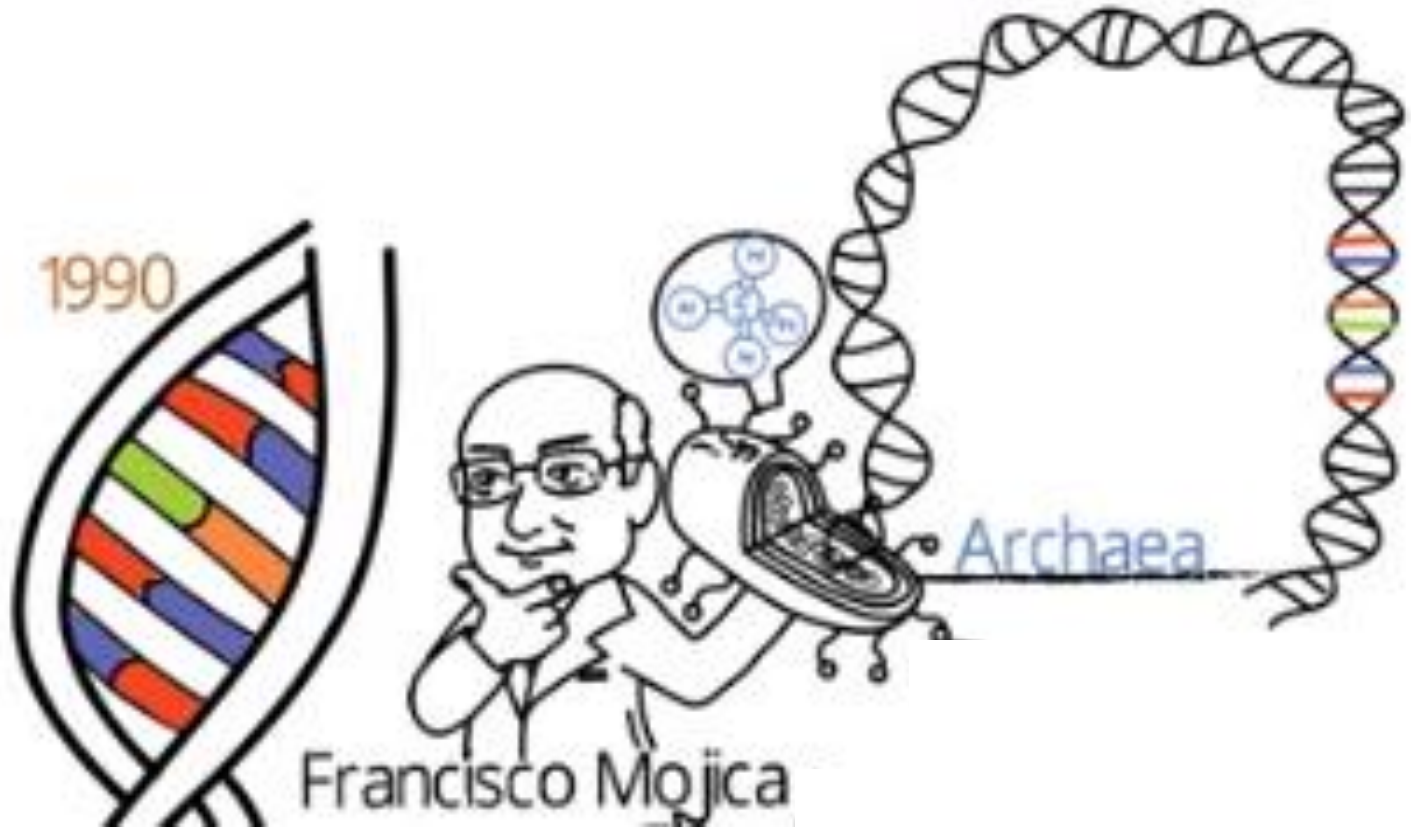
In the last paragraph of the Discussion…..

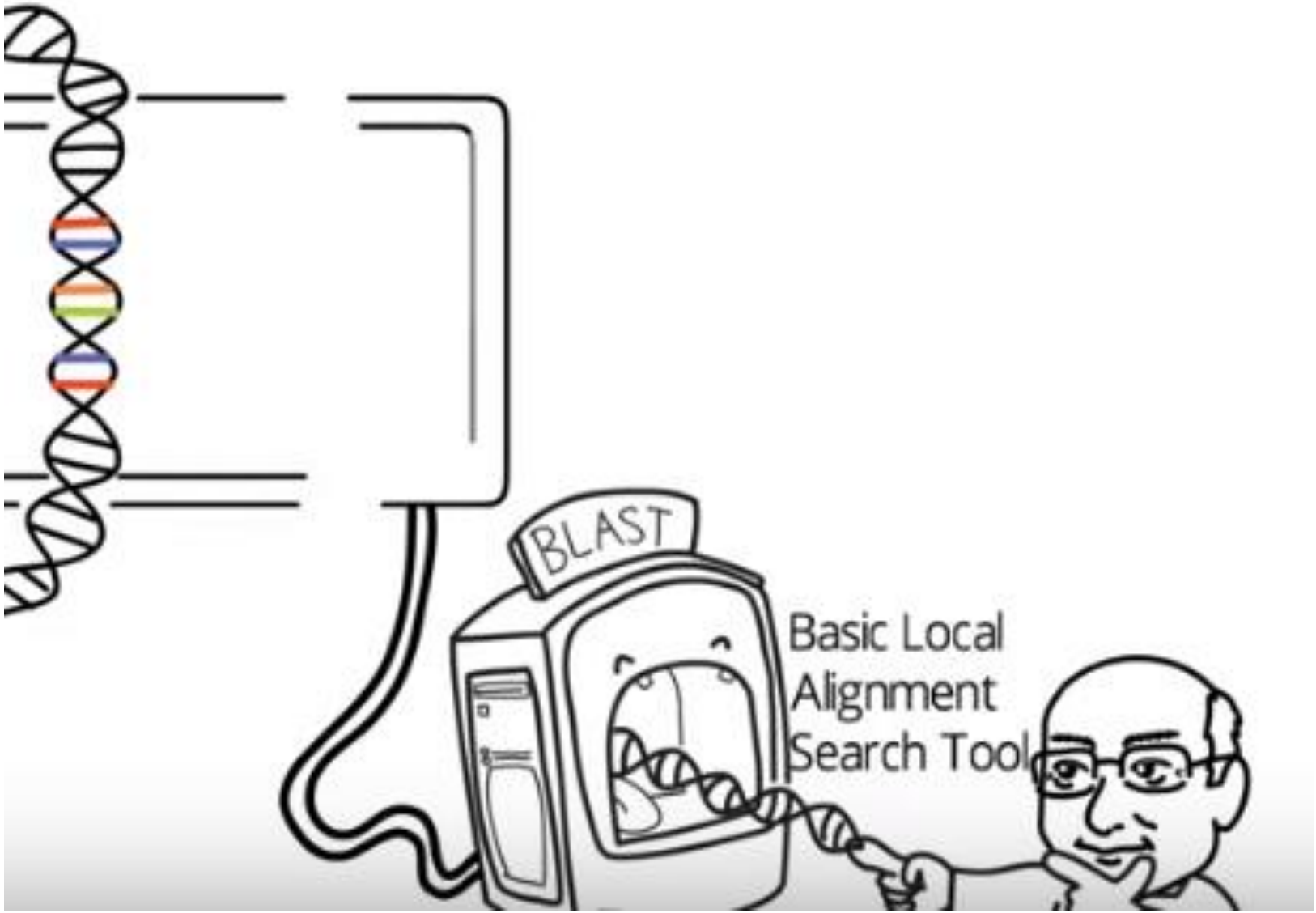
An unusual structure was found in the 3′-end flanking region of *iap* (Fig. 5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing. The first sequence was included in the putative transcriptional termination site and had less homology than 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.

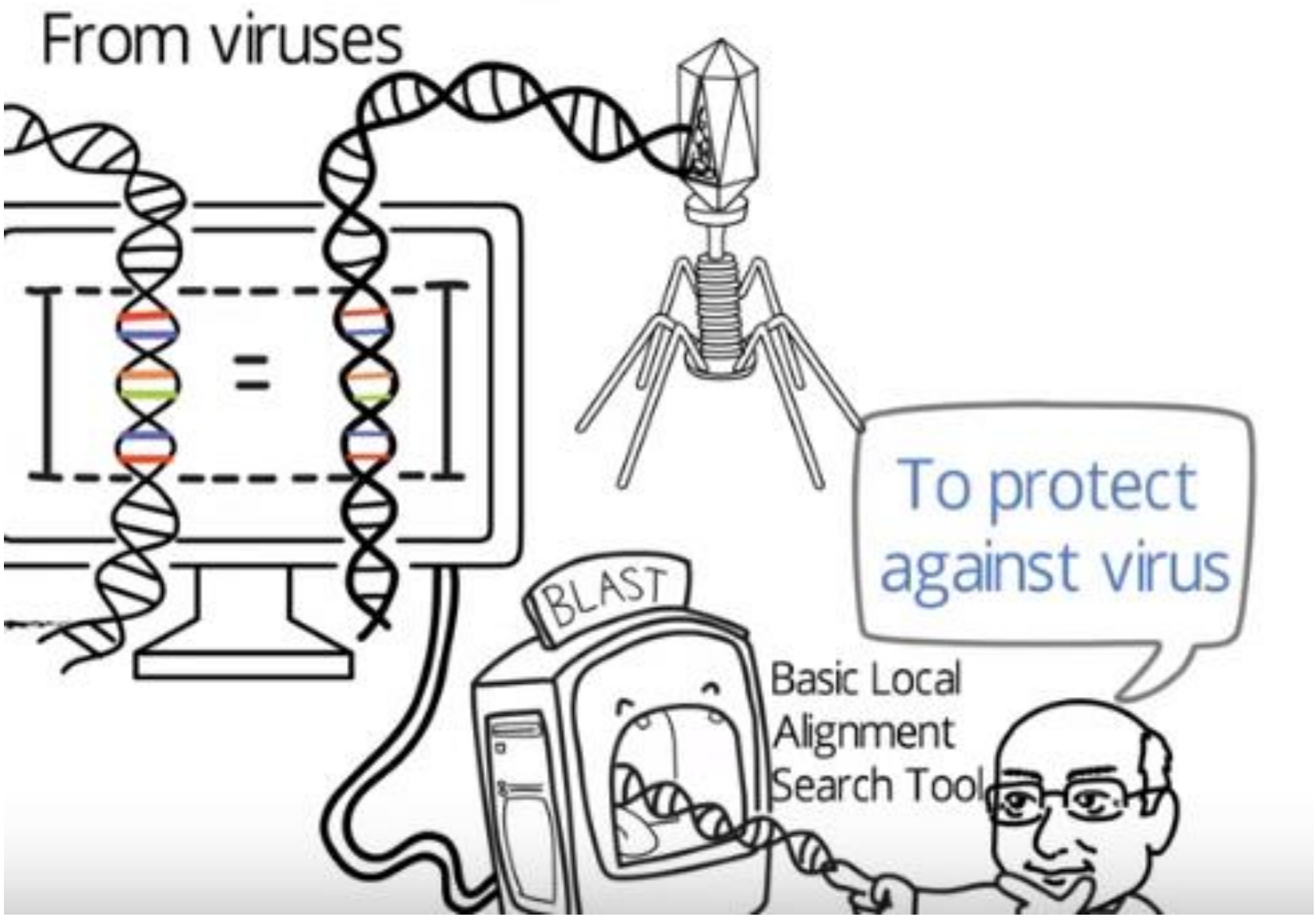
This is how science often happens…….

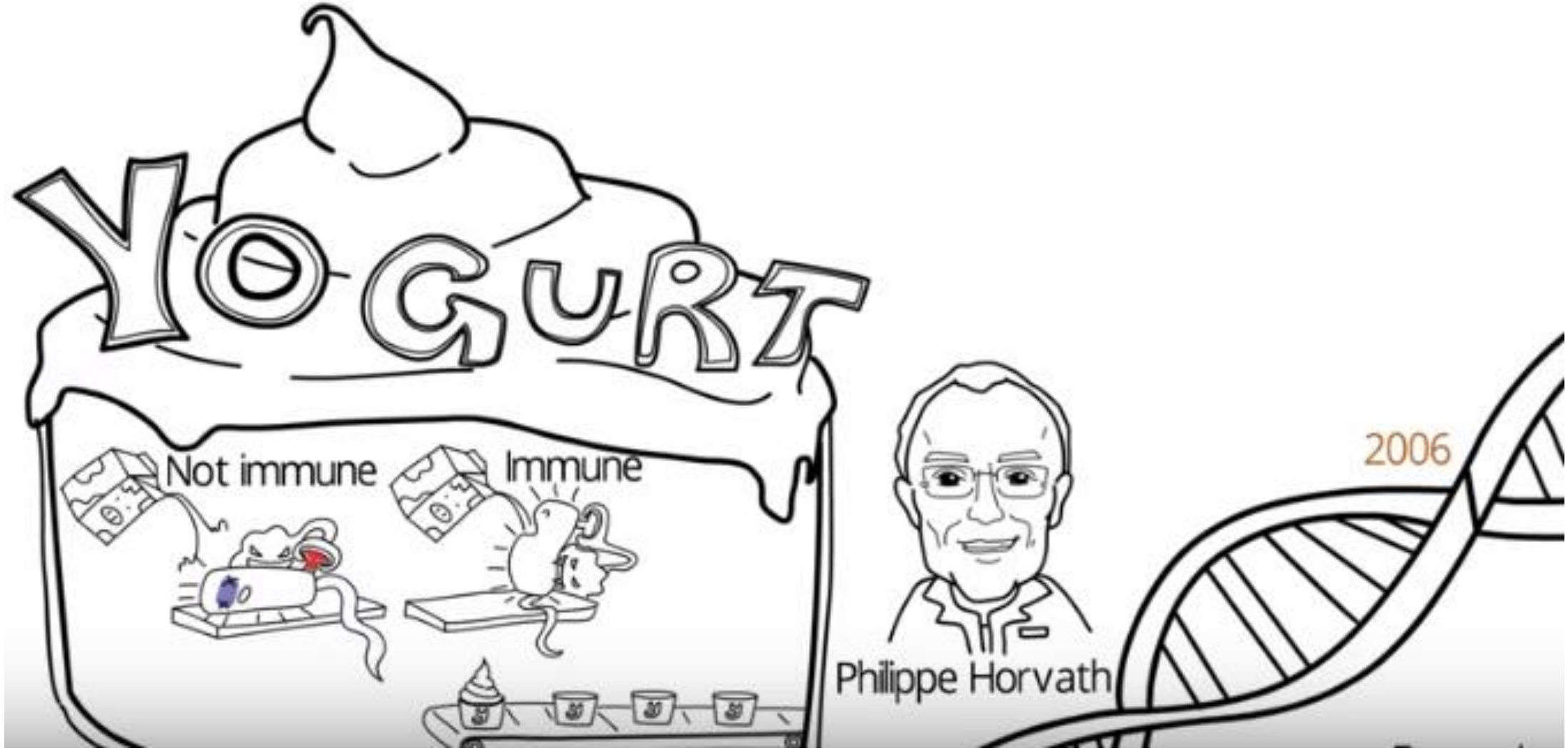
Named:

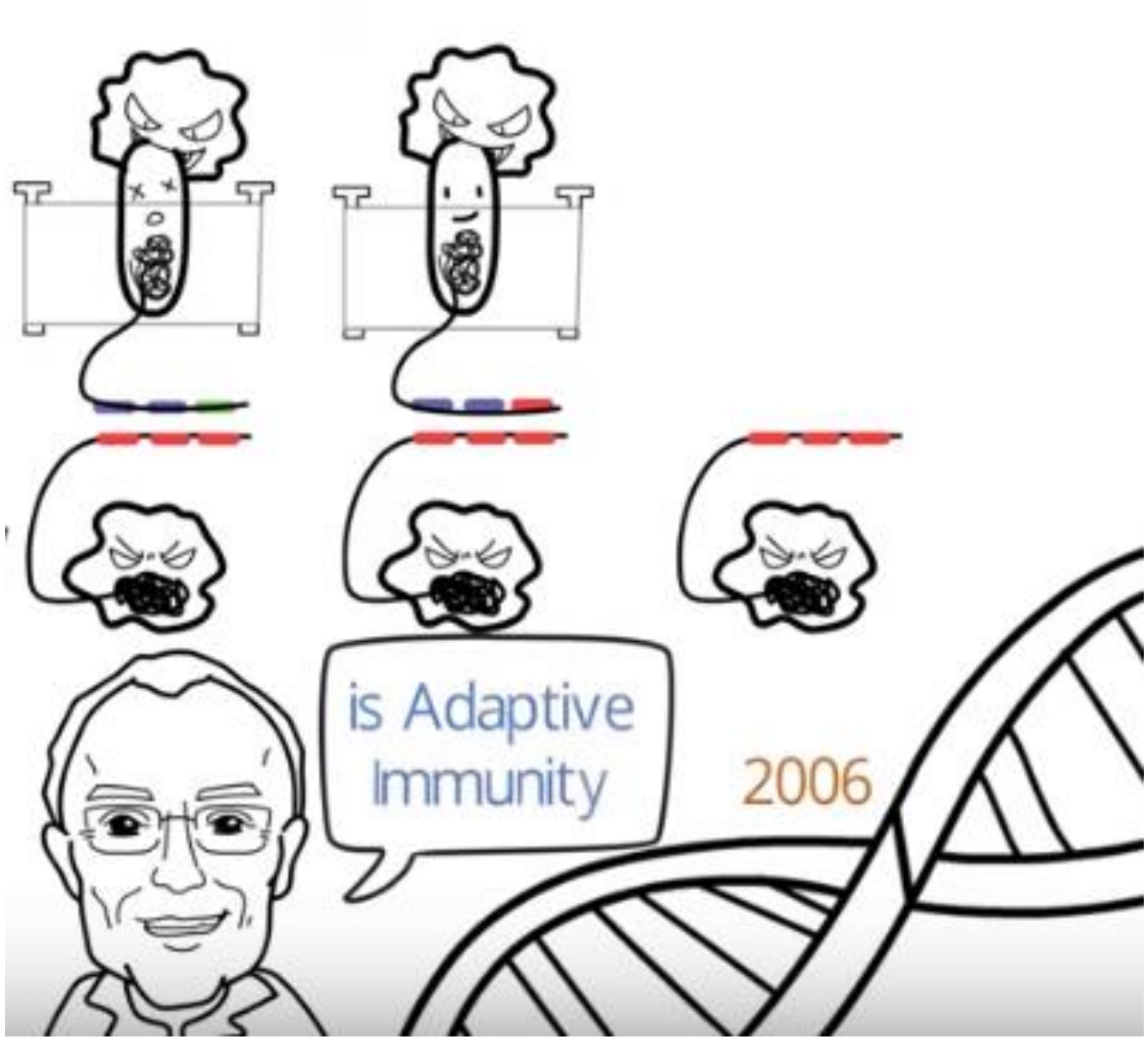
Clustered
Regularly
Interspaced
Short
Palindromic
Repeats



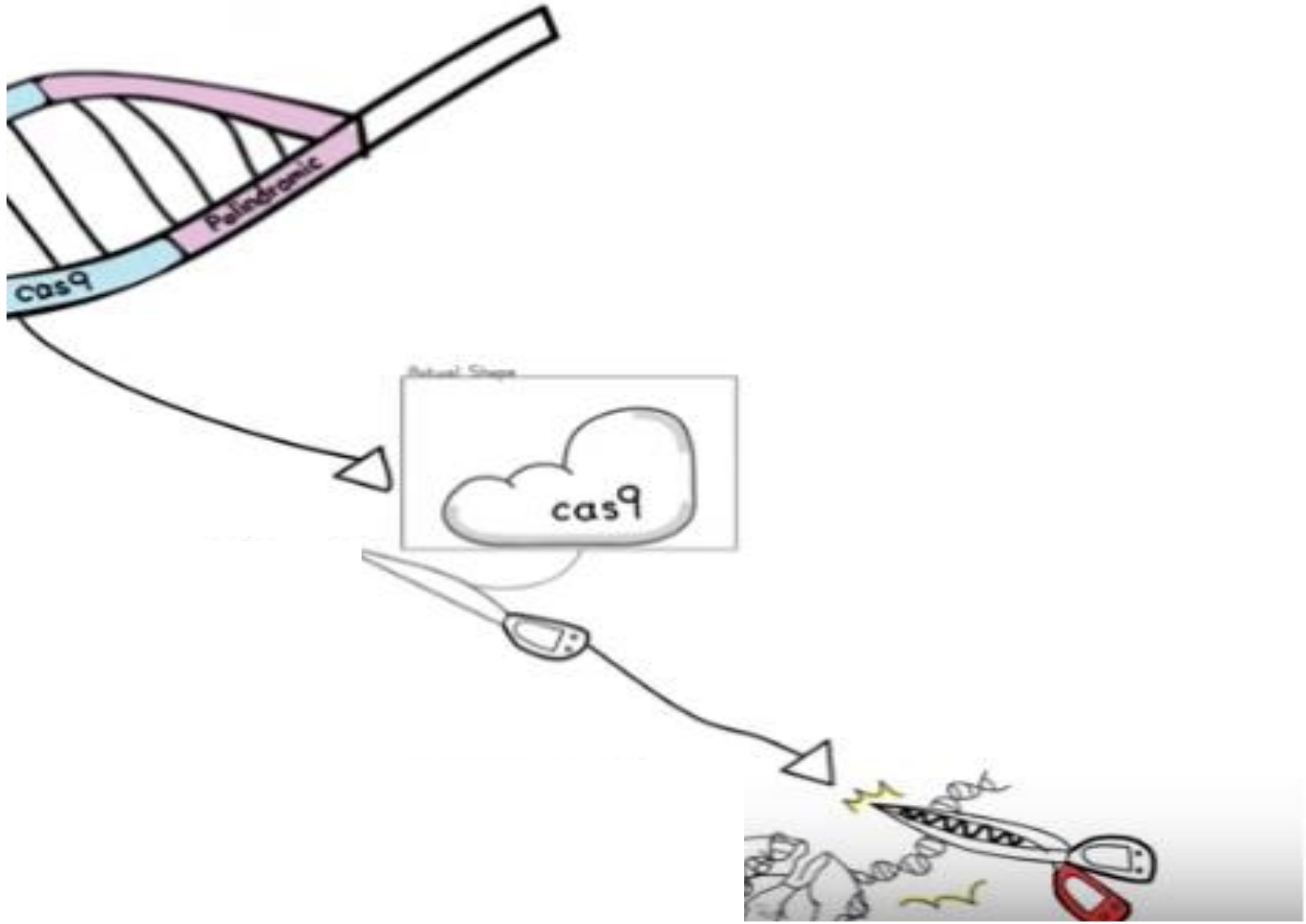

BLAST
Basic Local
Alignment
Search Tool

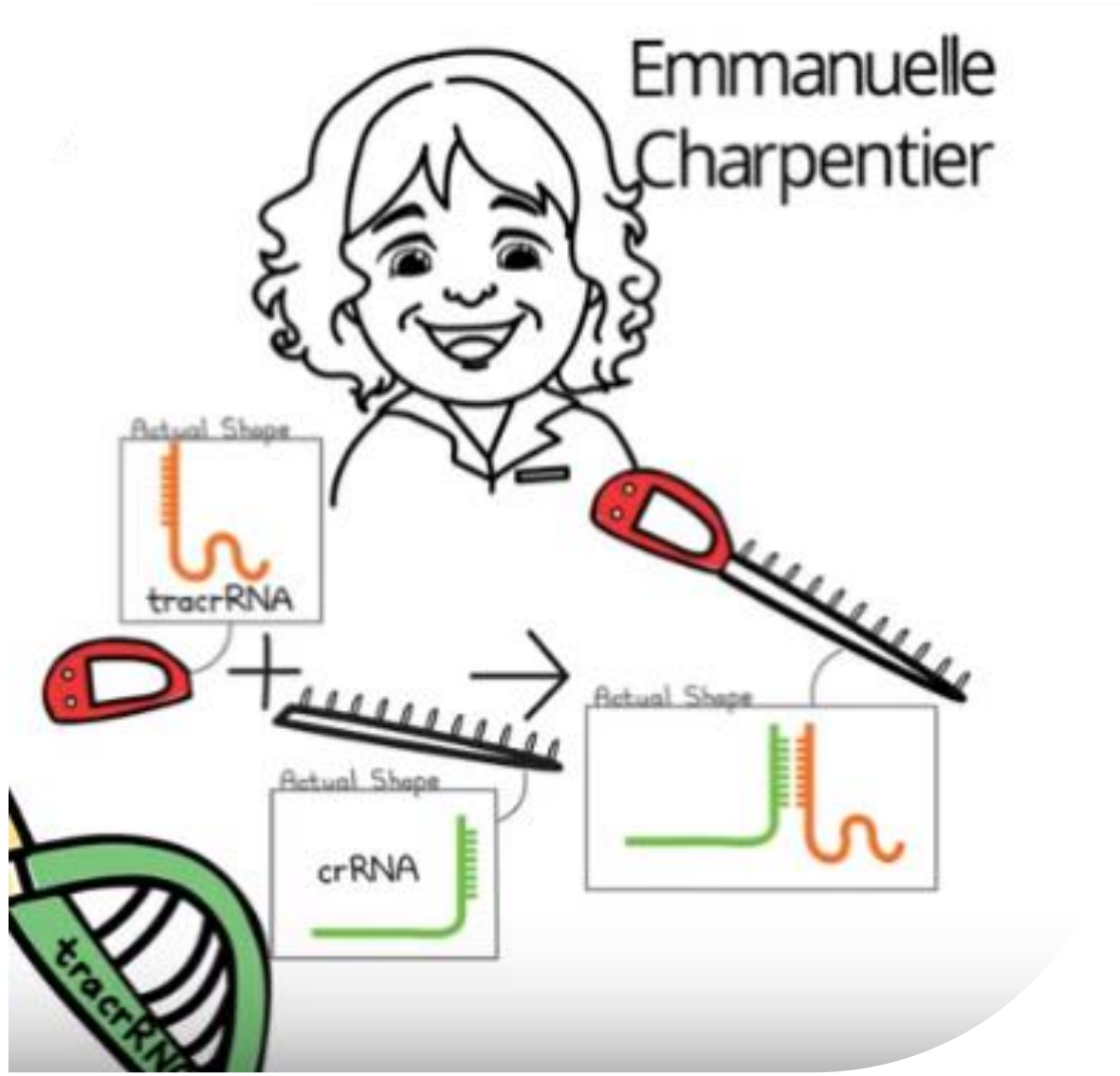

From viruses
To protect against virus
BLAST
Basic Local Alignment Search Tool


YOGURT
Not immune
Immune
Philippe Horvath
2006


is Adaptive Immunity
2006

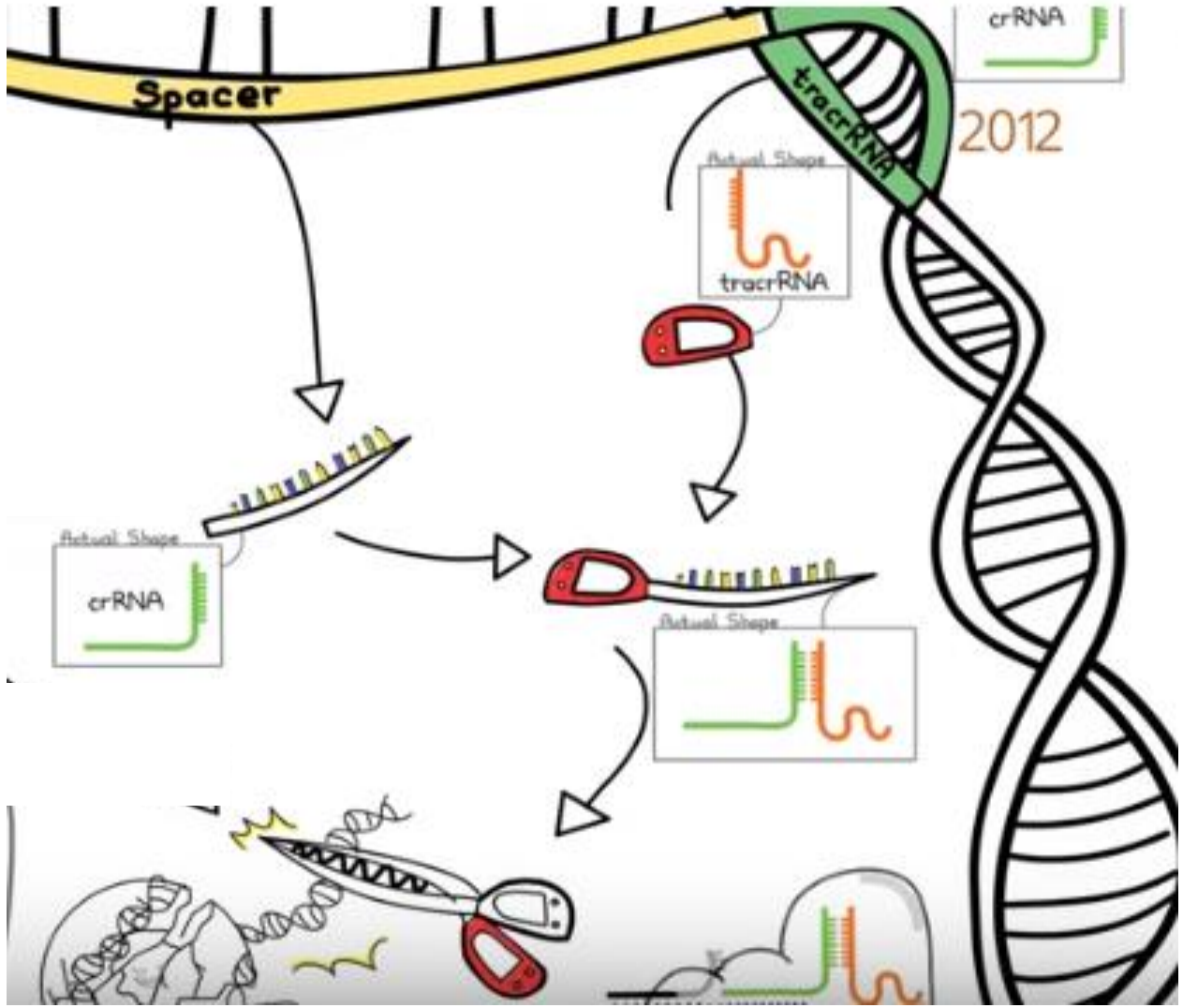
Cas-9 discovery:




Emmanuelle
Charpentier
Actual Shape
tracrRNA
Actual Shape
crRNA
Actual Shape

Full component of crispr:

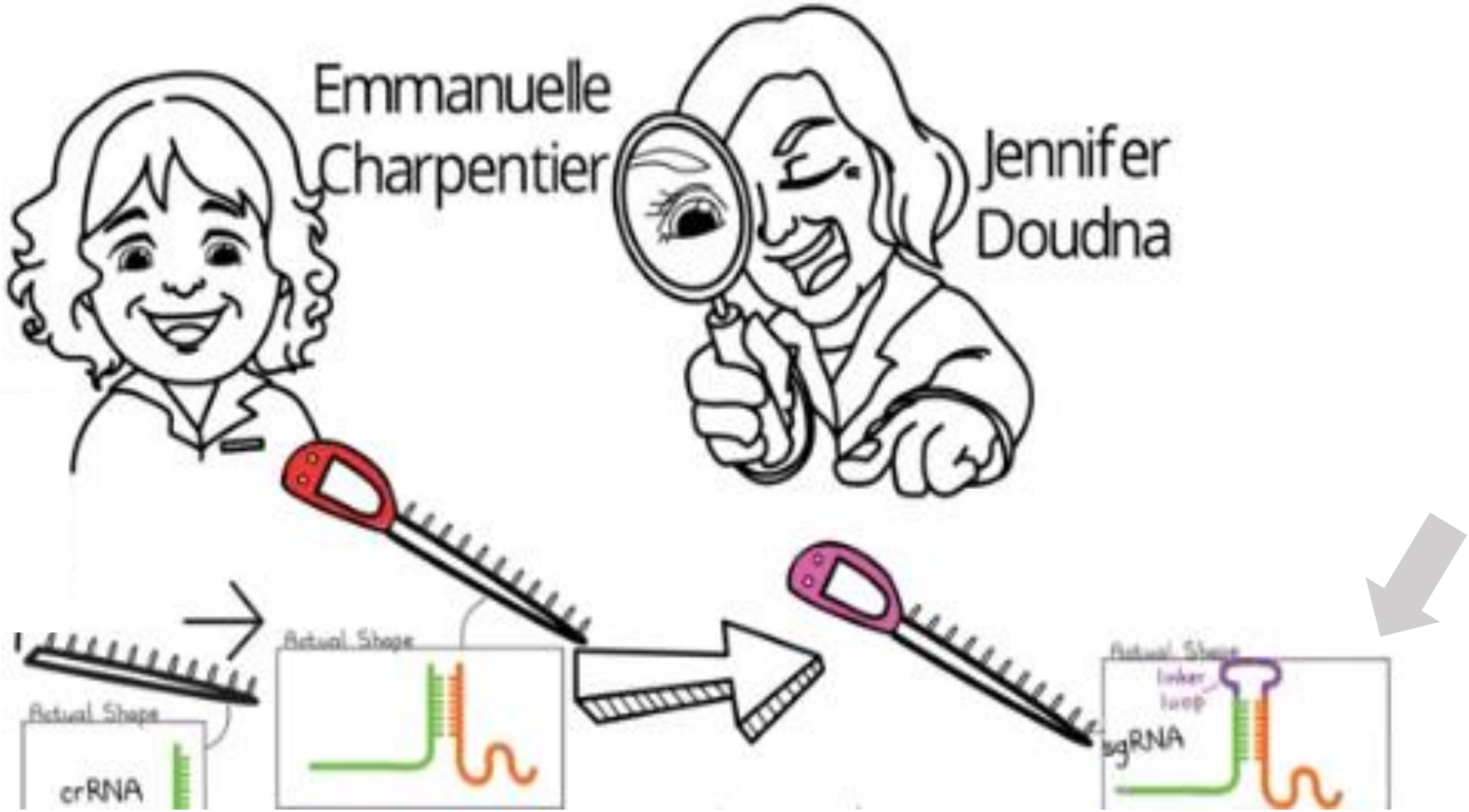
- Spacer
- Trac RNA
- Cas-9

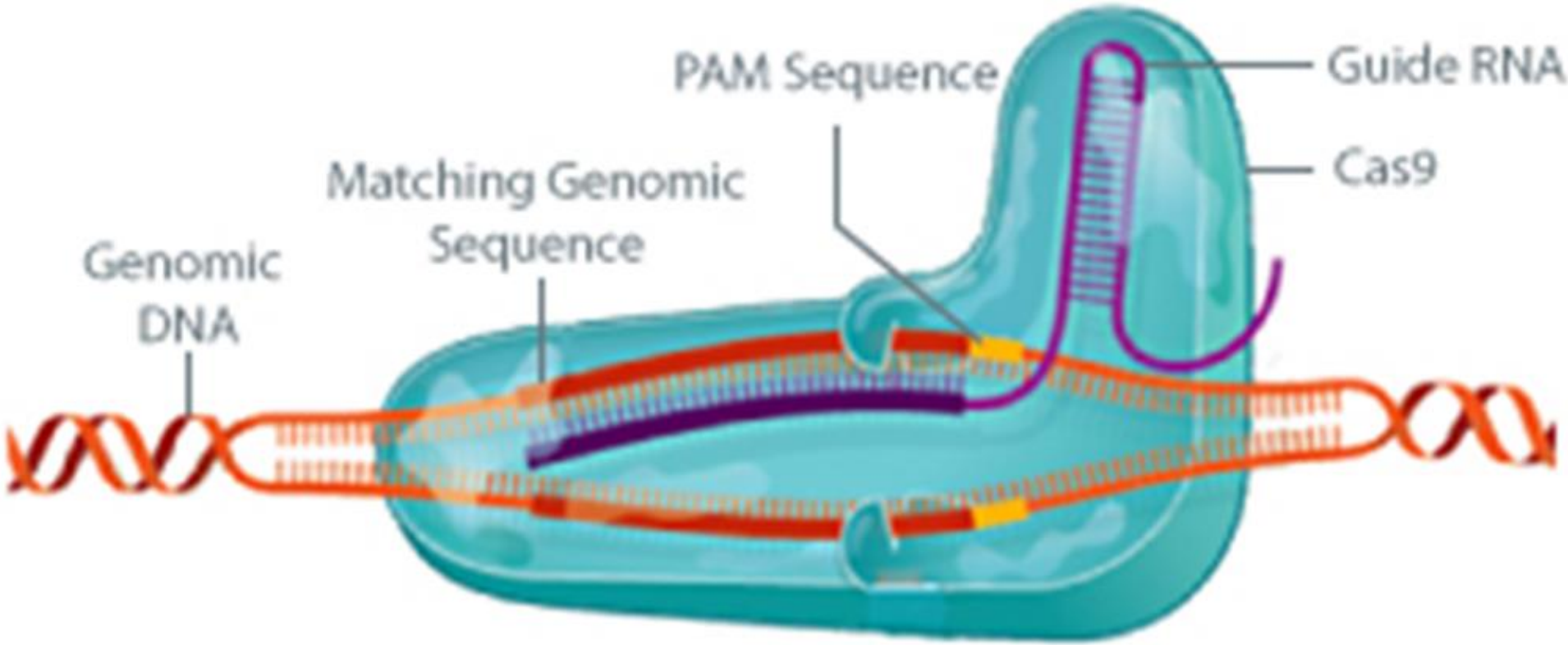



2013
Accelerated
tinkering
RISING STAR!!!

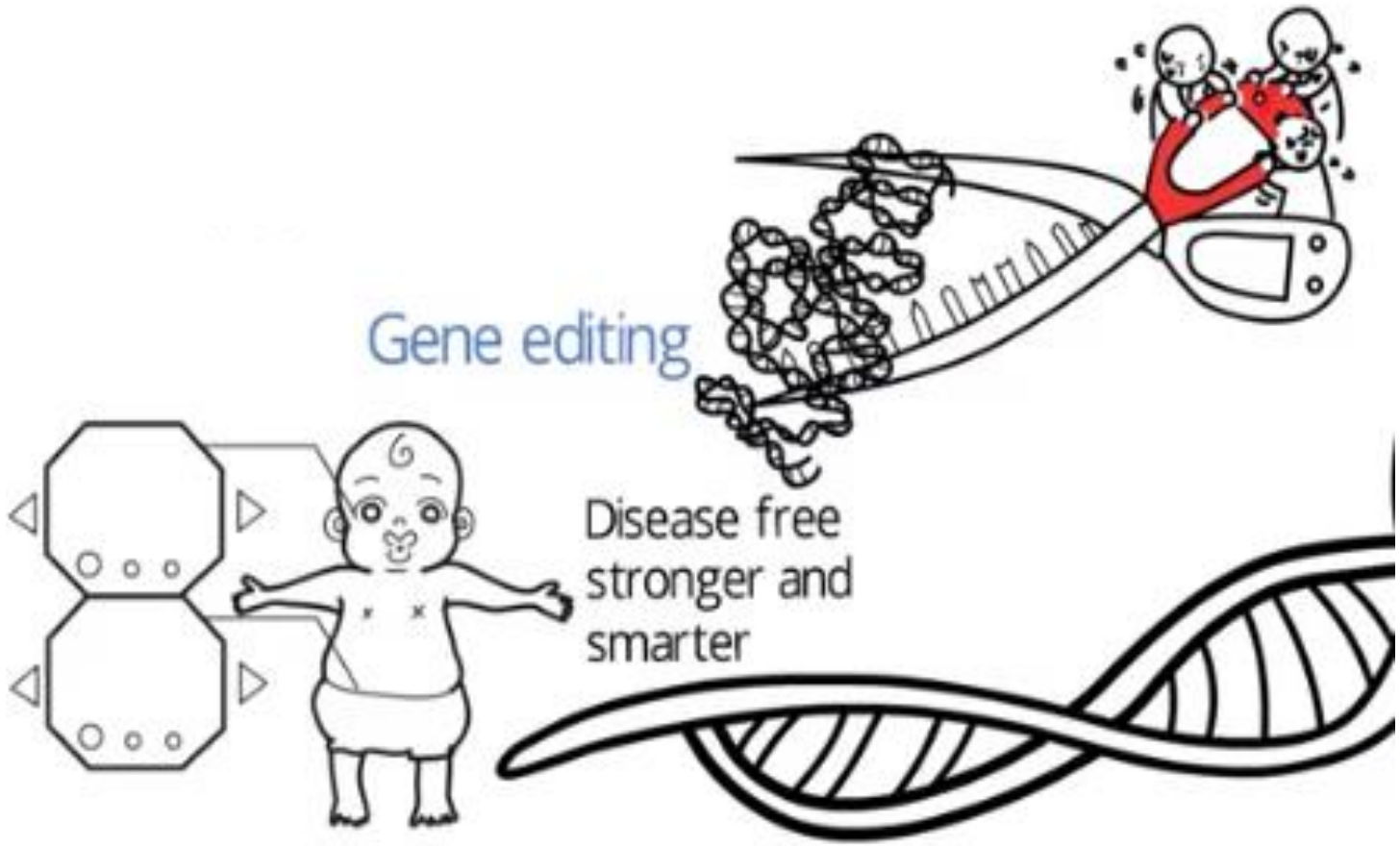

Virginlijus
Siksnys
In-vivo
In-vitro

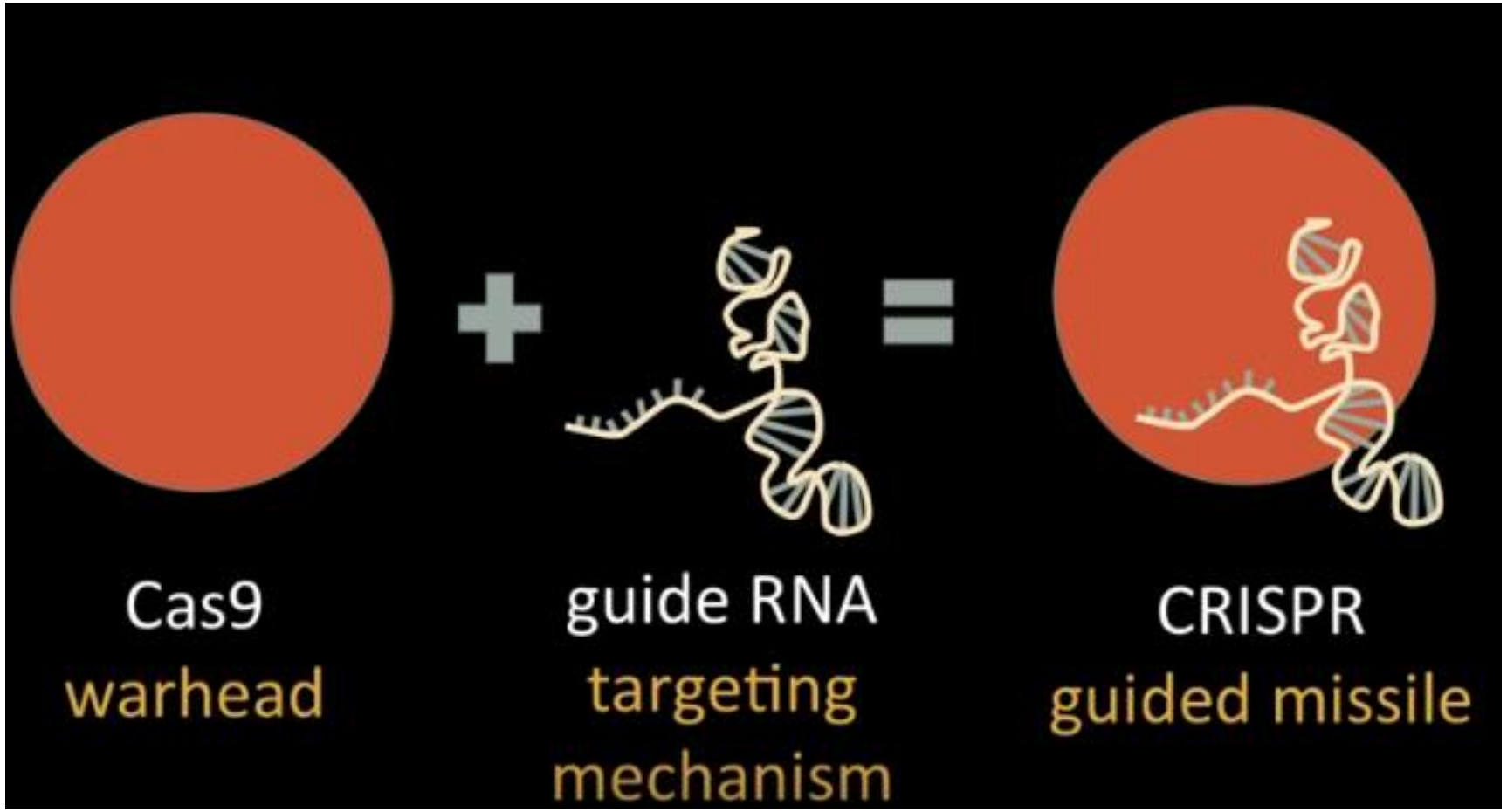
Optimized natural pathway



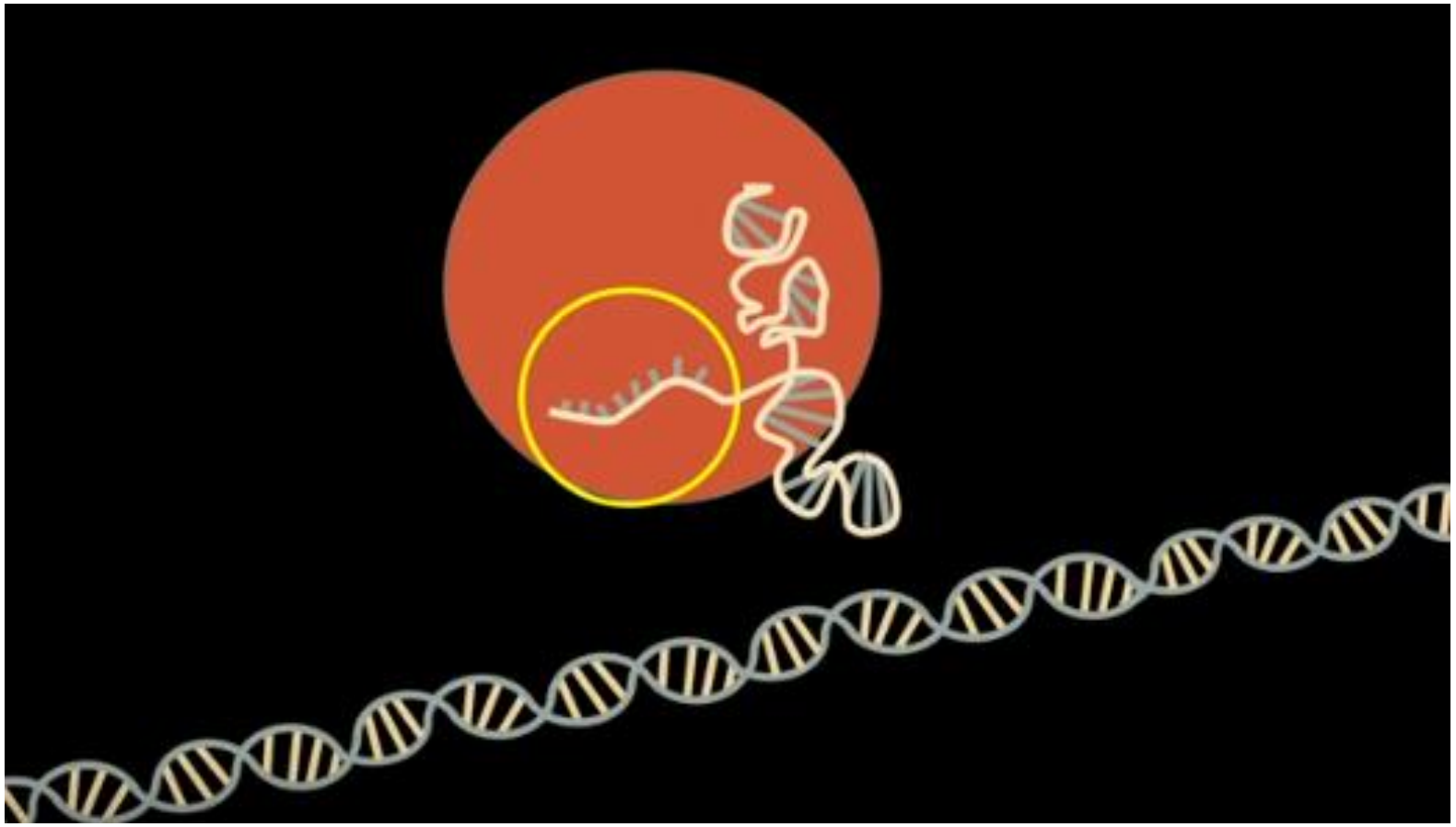

PAM Sequence
Guide RNA
Cas9
Matching Genomic Sequence
Genomic DNA

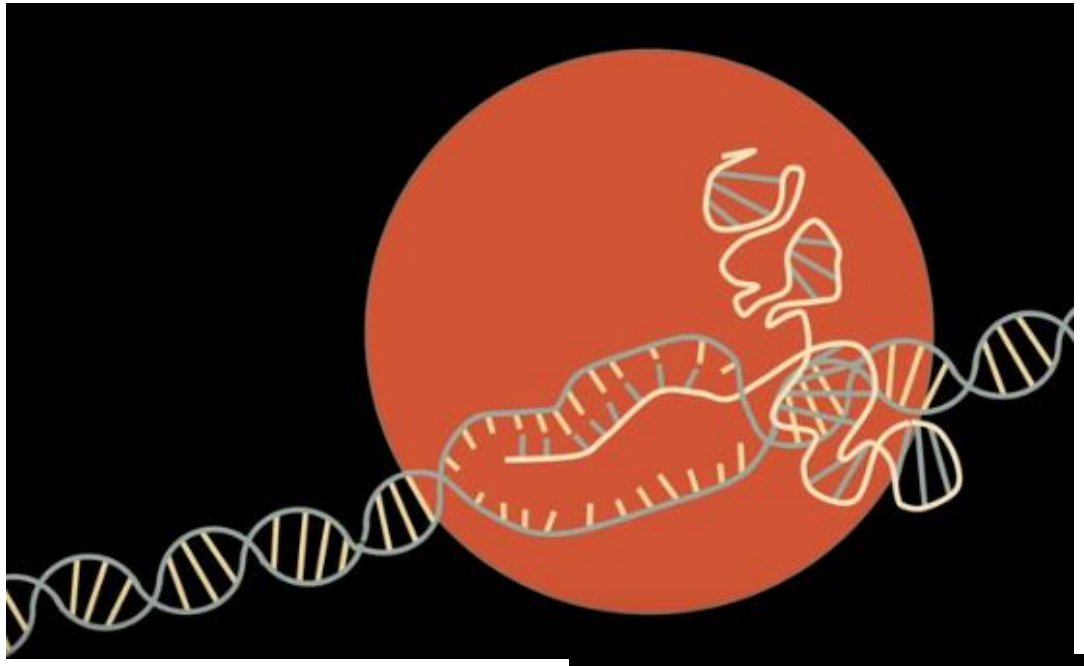

2013
Feng Zhang
Optimized for mammalian gene

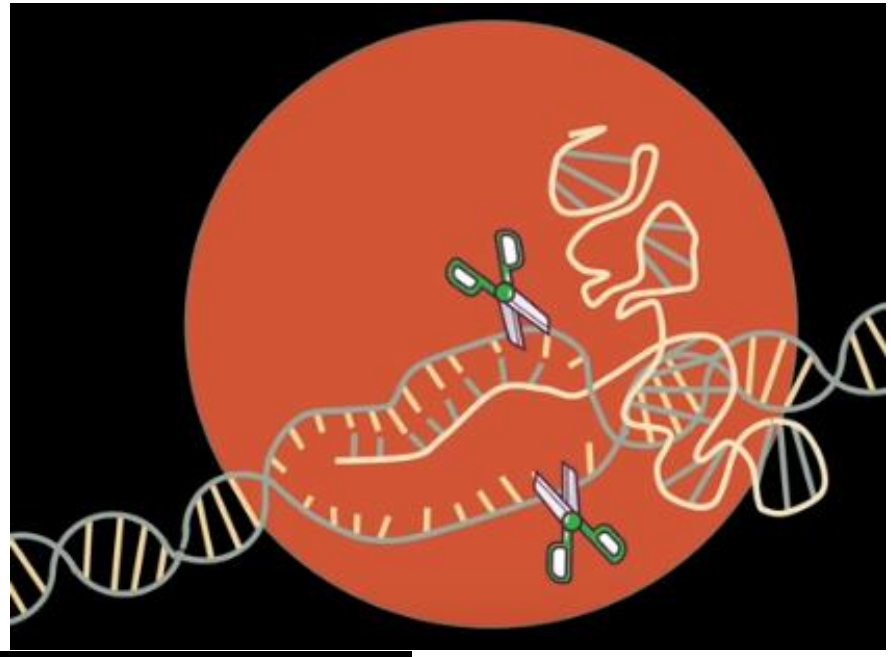

Gene editing
Disease free
stronger and
smarter


Cas9
warhead
guide RNA
targeting
mechanism
CRISPR
guided missile

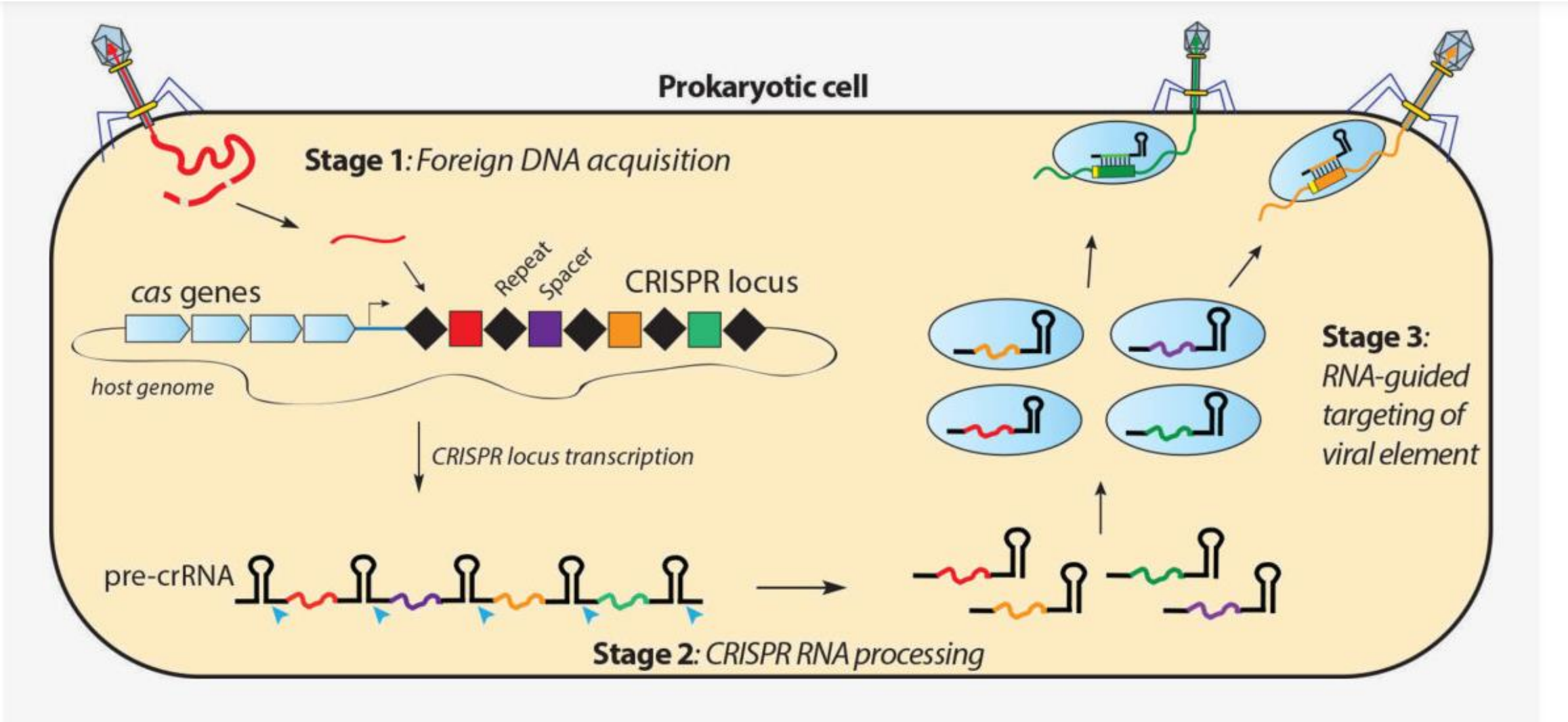
- Easy to design
- Cheap to buy
- But still needs professional labs.



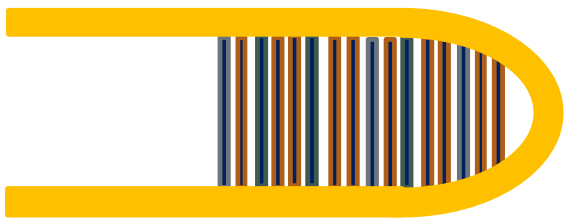




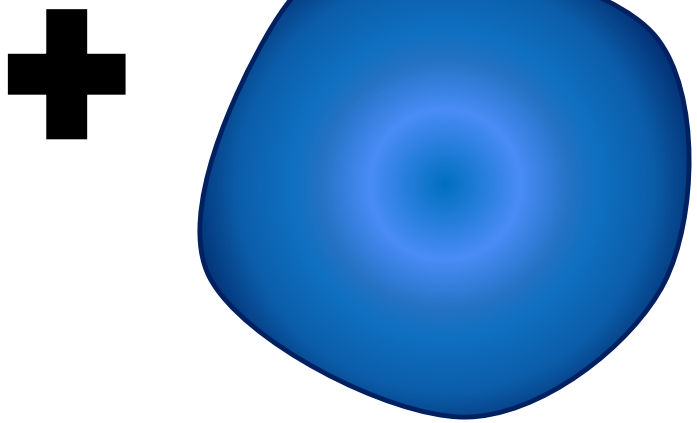

PANIC


Prokaryotic cell
Stage 1: Foreign DNA acquisition
cas genes
Repeat
Spacer
CRISPR locus
host genome
CRISPR locus transcription
pre-crRNA
Stage 2: CRISPR RNA processing
Stage 3: RNA-guided targeting of viral element

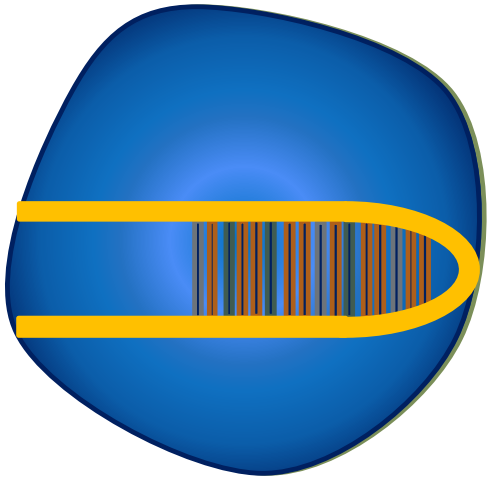
TO INITIATE GENE MODIFICATION



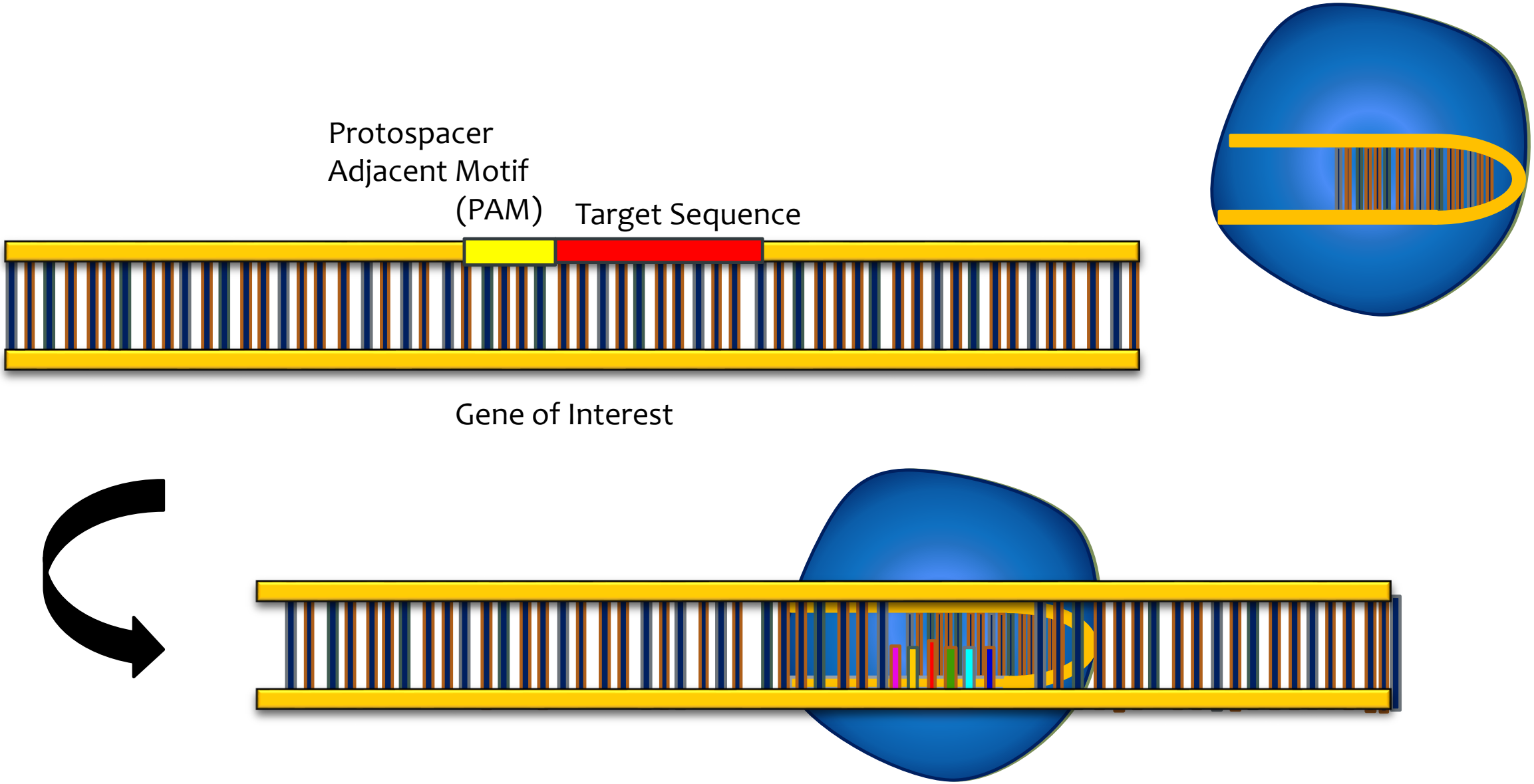
sgRNA
(single guide RNA)

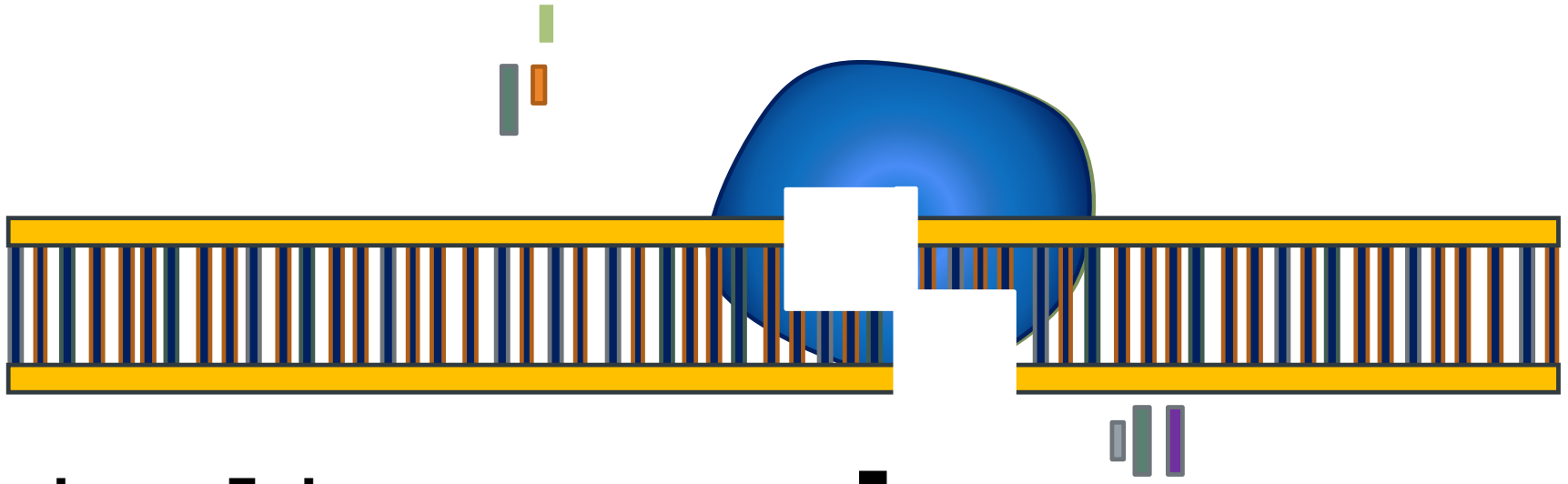


Cas9 nuclease

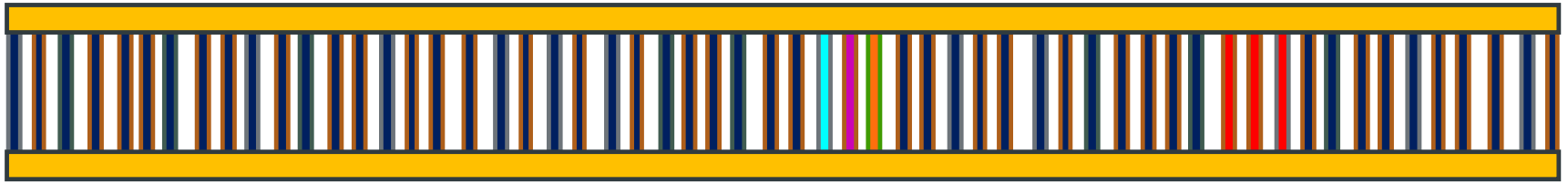


Cas9 complex

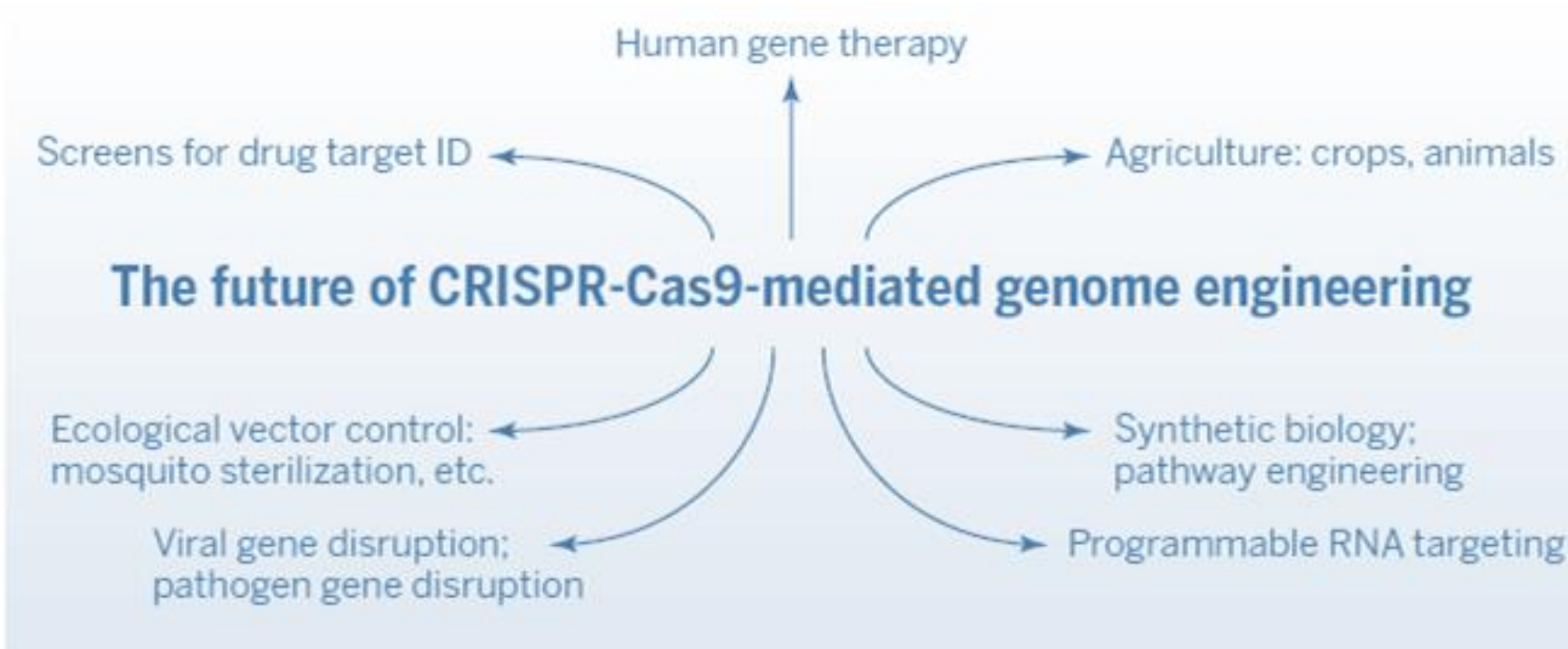

Protospacer
Adjacent Motif
(PAM)
Target Sequence
Gene of Interest



Non-Homologous End Joining (NHEJ) DNA repair pathway



Stop Codon


Human gene therapy
Screens for drug target ID
Agriculture: crops, animals
The future of CRISPR-Cas9-mediated genome engineering
Ecological vector control:
mosquito sterilization, etc.
Synthetic biology;
pathway engineering
Viral gene disruption;
pathogen gene disruption
Programmable RNA targeting