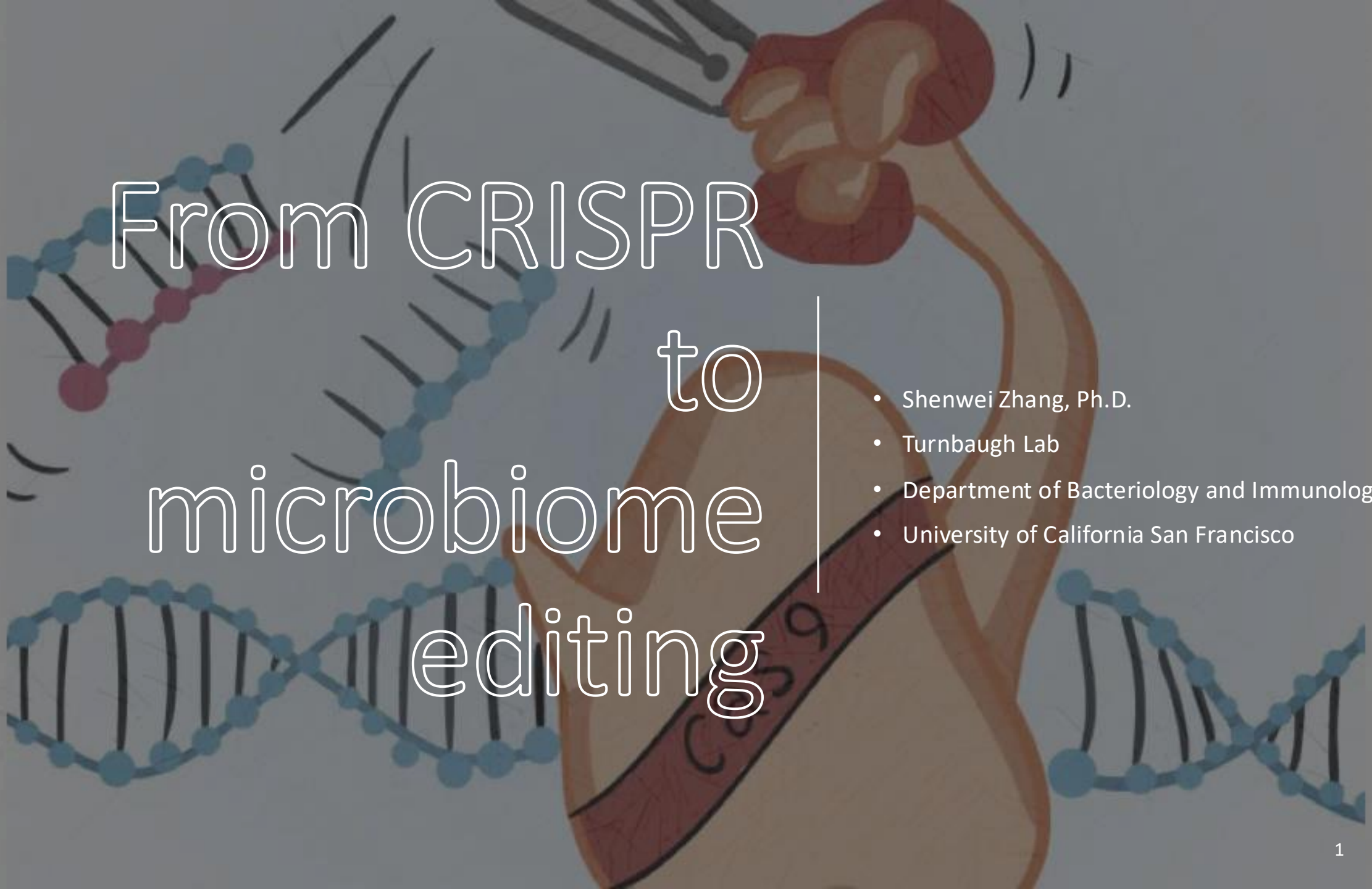
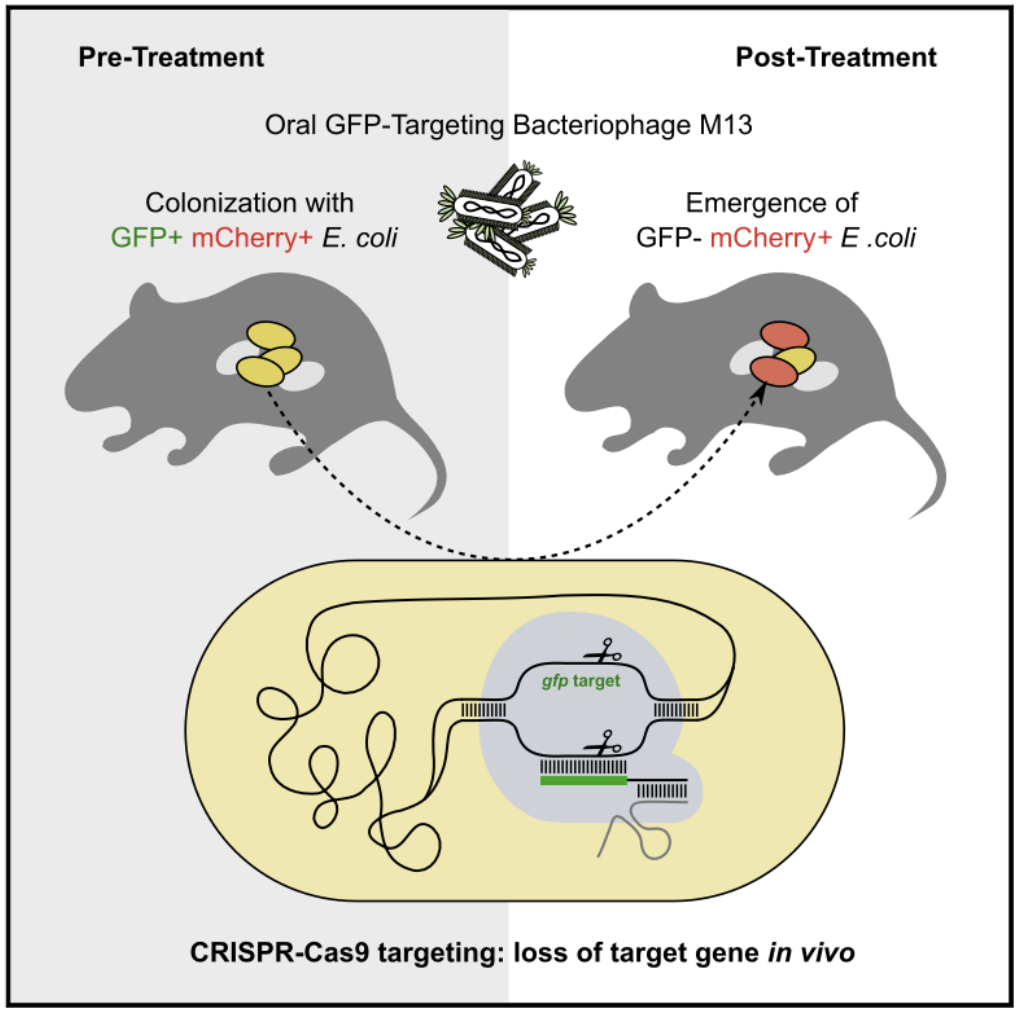


From CRISPR to microbiome editing

- Shenwei Zhang, Ph.D.
- Turnbaugh Lab
- Department of Bacteriology and Immunology
- University of California San Francisco

Phage-delivered CRISPR-Cas9 for strain-specific depletion and genomic deletions in the gut microbiome

Graphical abstract



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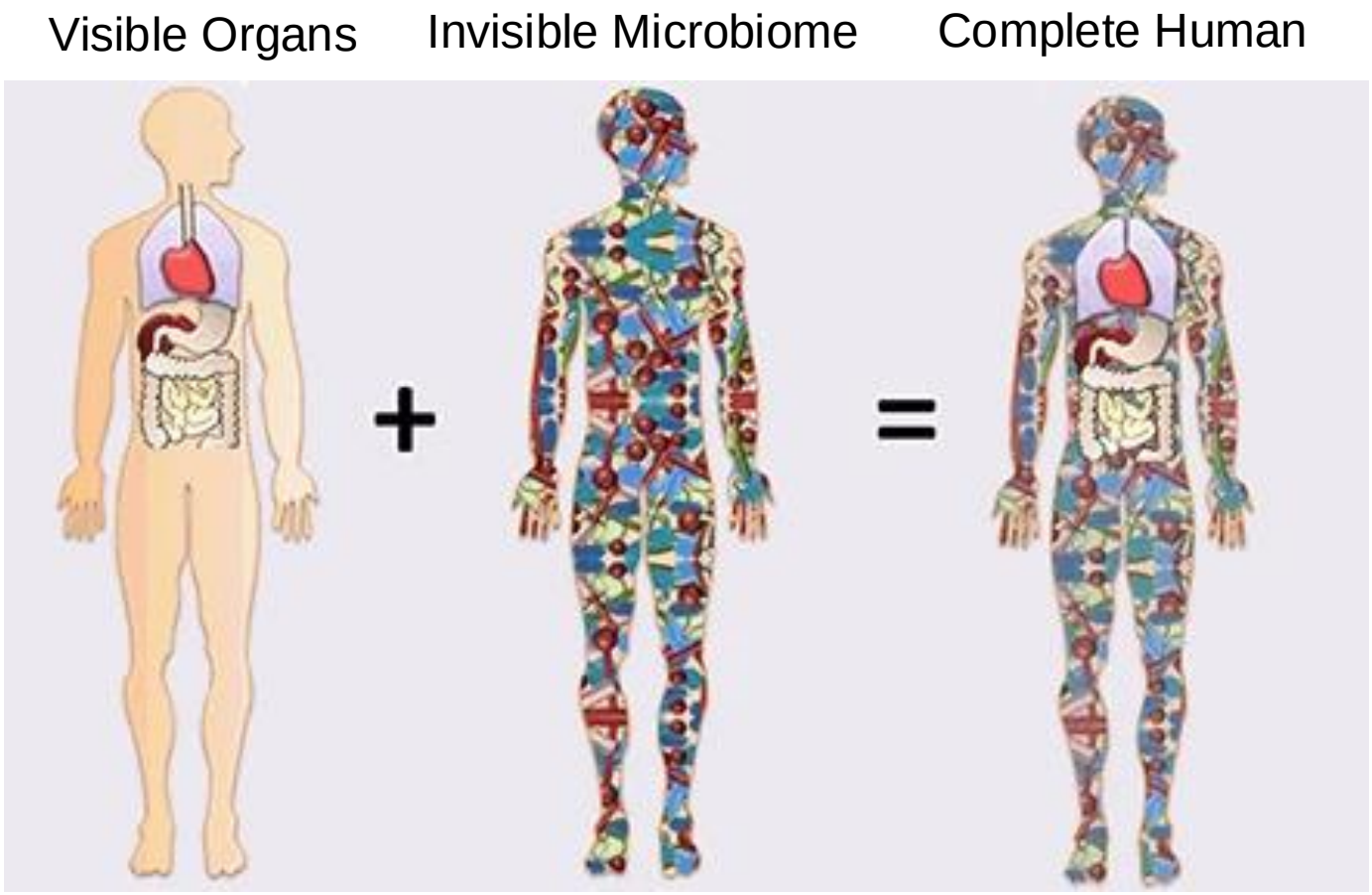
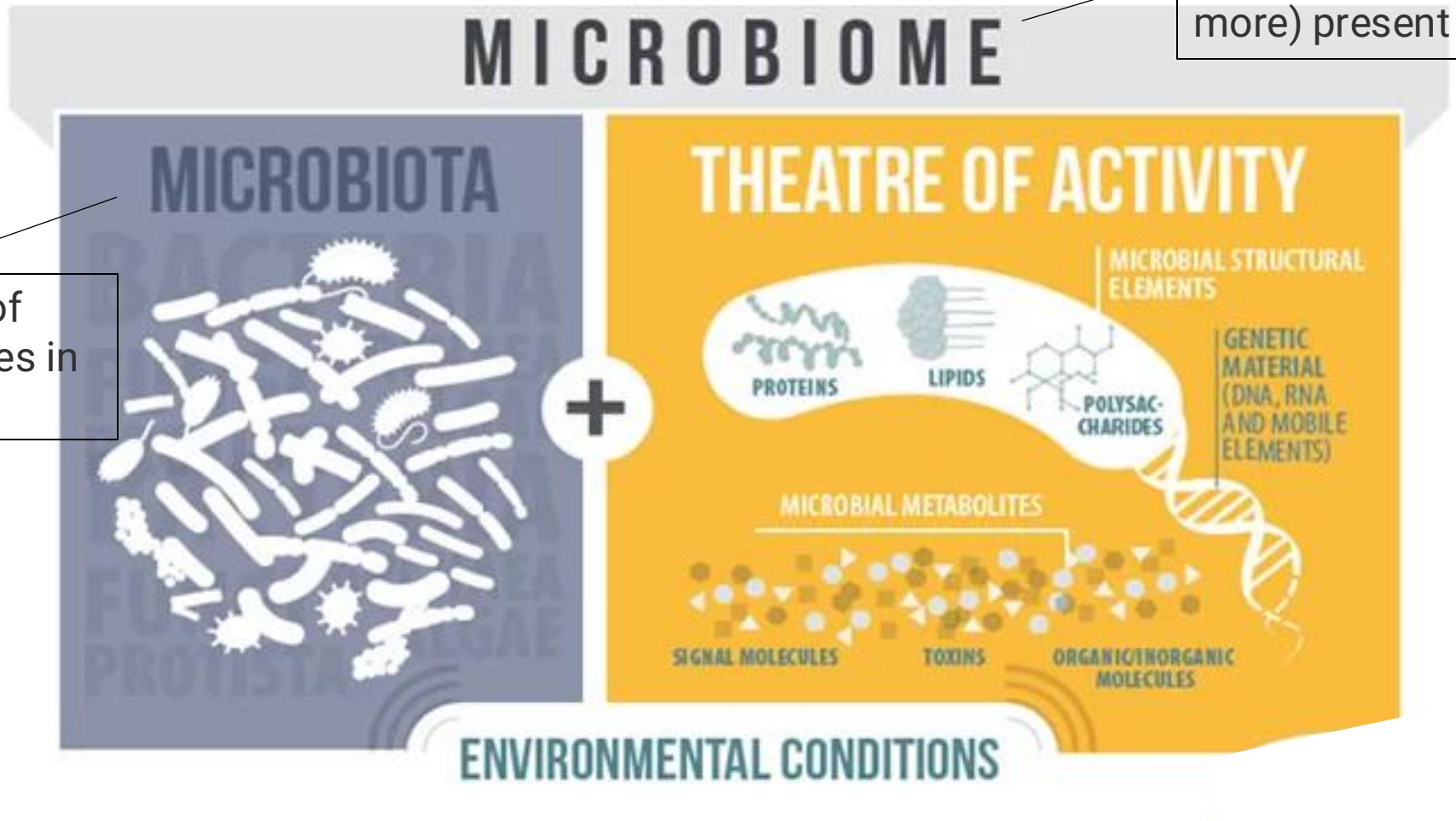
Human microbiome: an important invisible “organ”



Microbiota vs. Microbiome

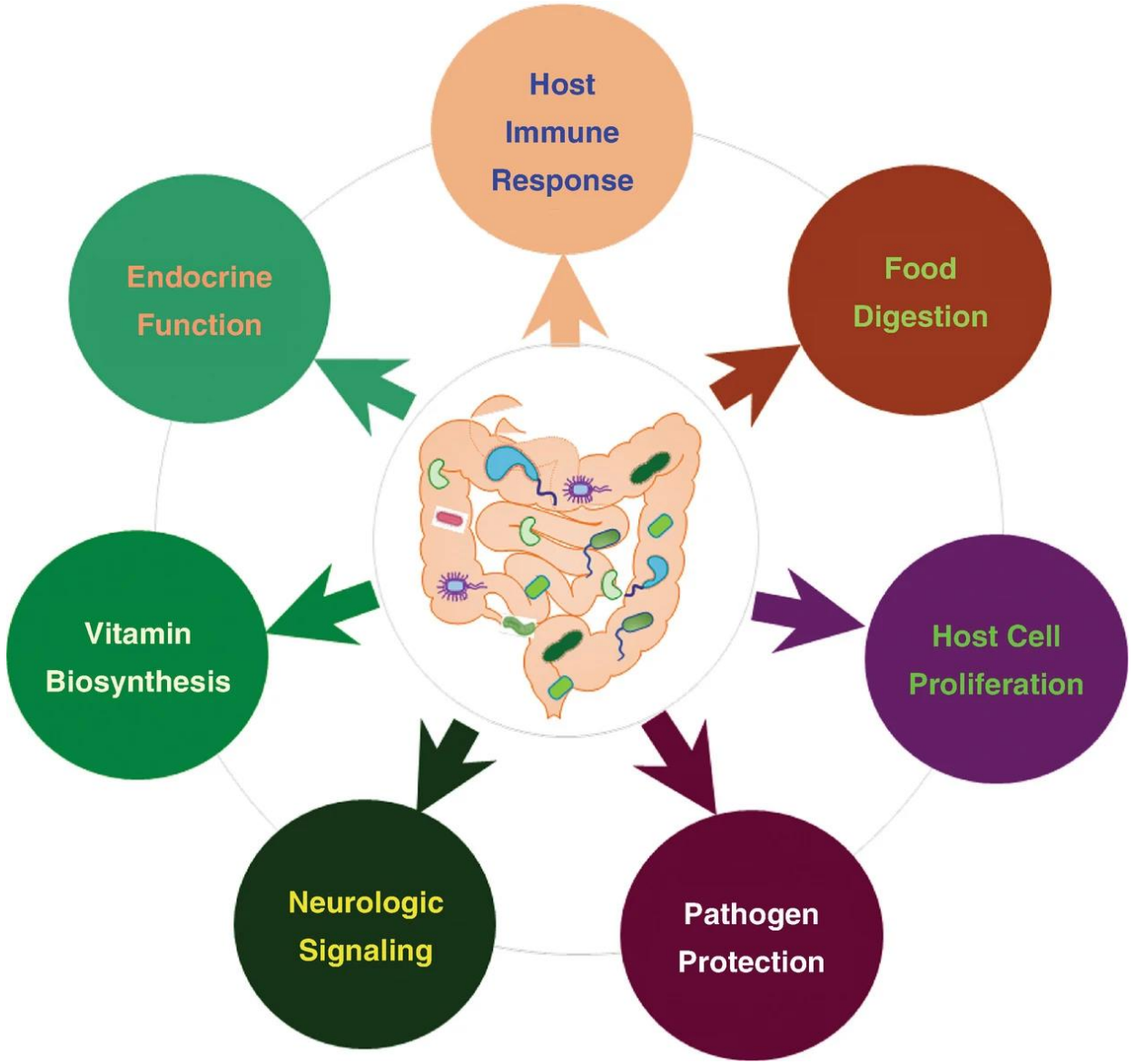
The aggregate collection of genomes and genes (and more) present in a microbiota.



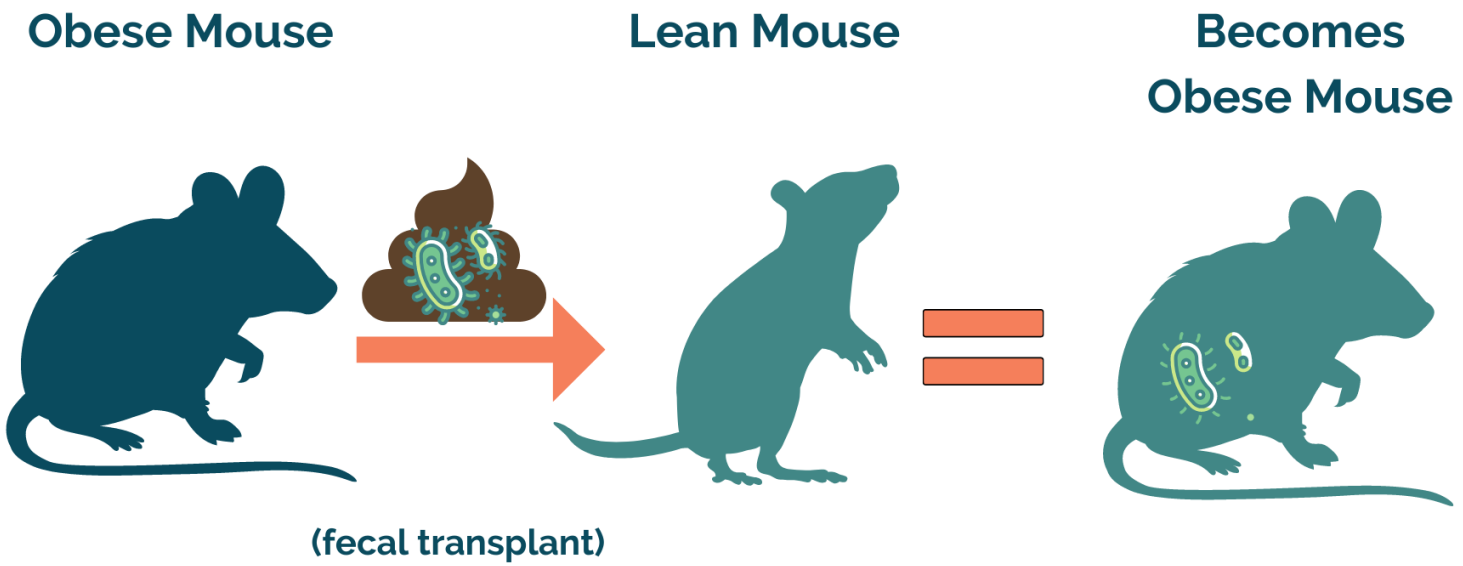
The community of microbes that lives in an individual.

Scanning electron micrograph (SEM) of human small intestine microbiota.

Functions of the gut microbiota

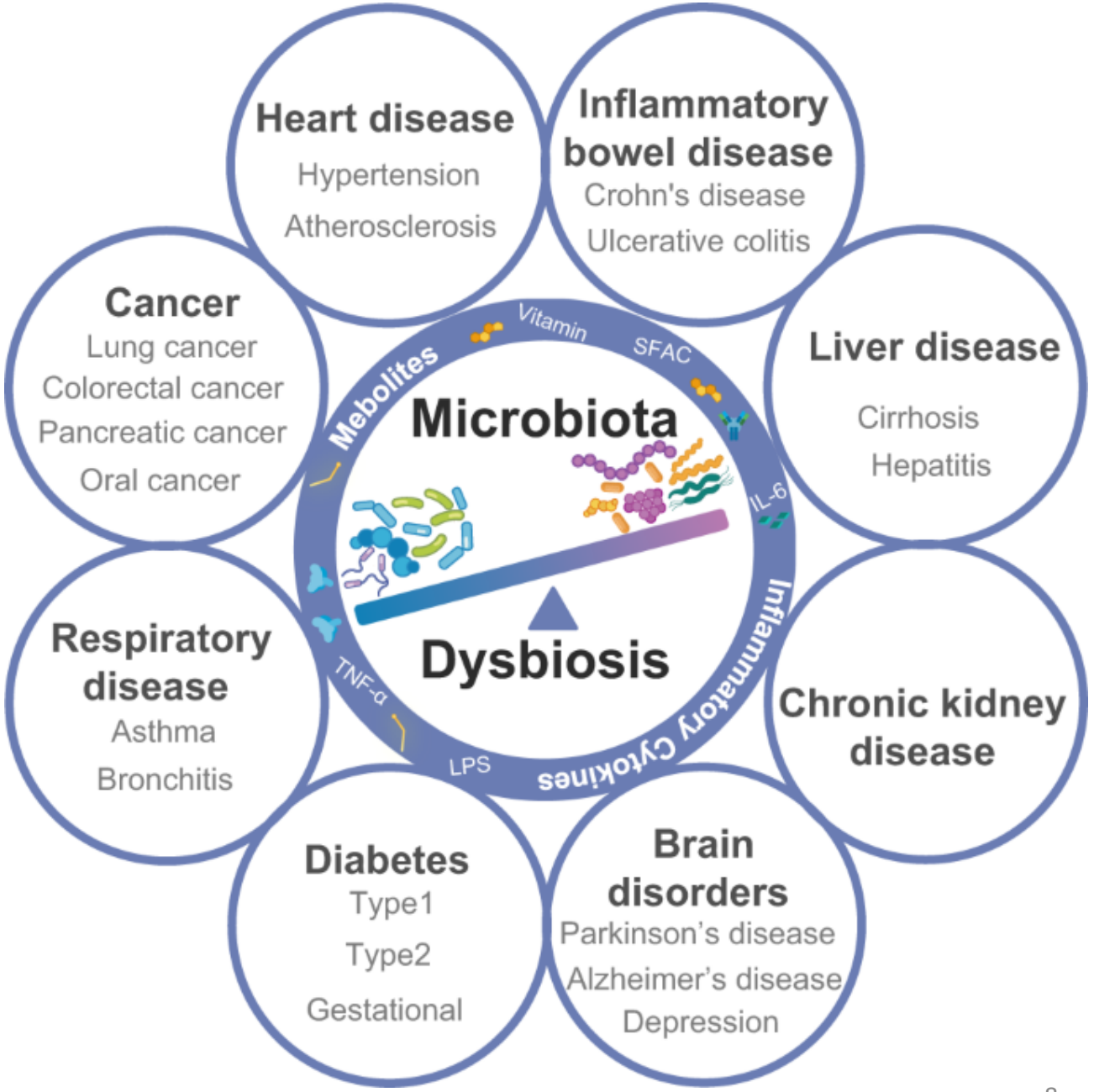


Gut microbiota contributes to the pathophysiology of obesity

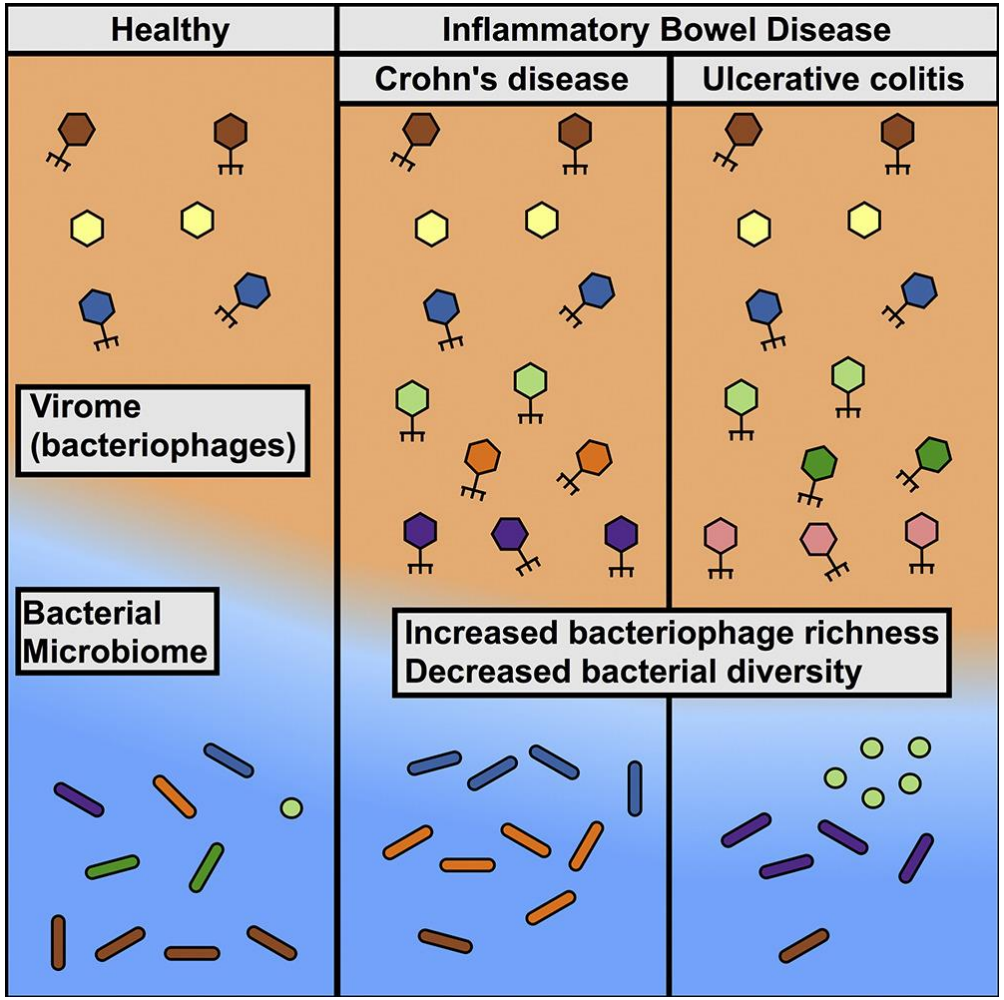


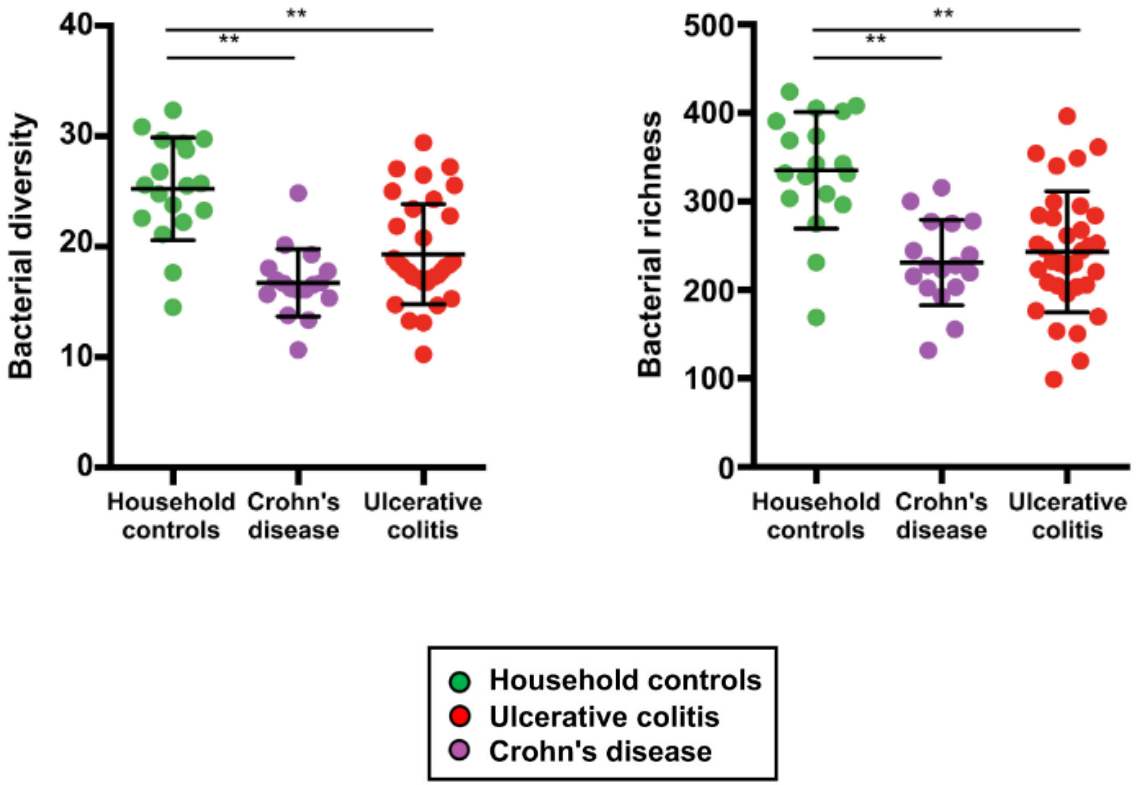
Turnbaugh *et al*., 2016.

Human microbiota dysbiosis contributes to various diseases



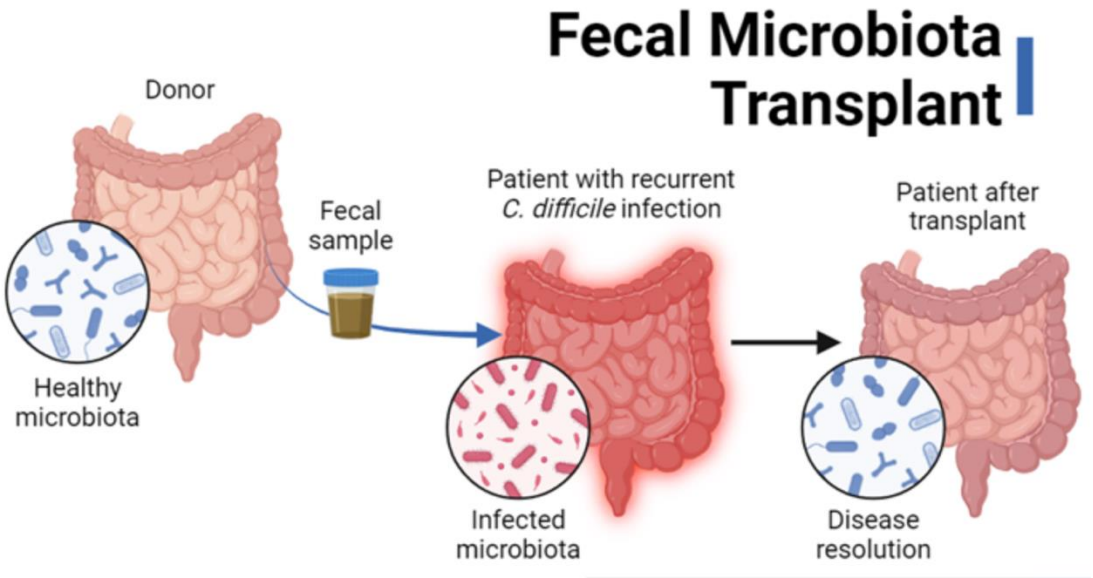
Inflammatory bowel disease (IBD):
decrease in bacterial diverity and richness





Norman et al., (2015) *Cell.*

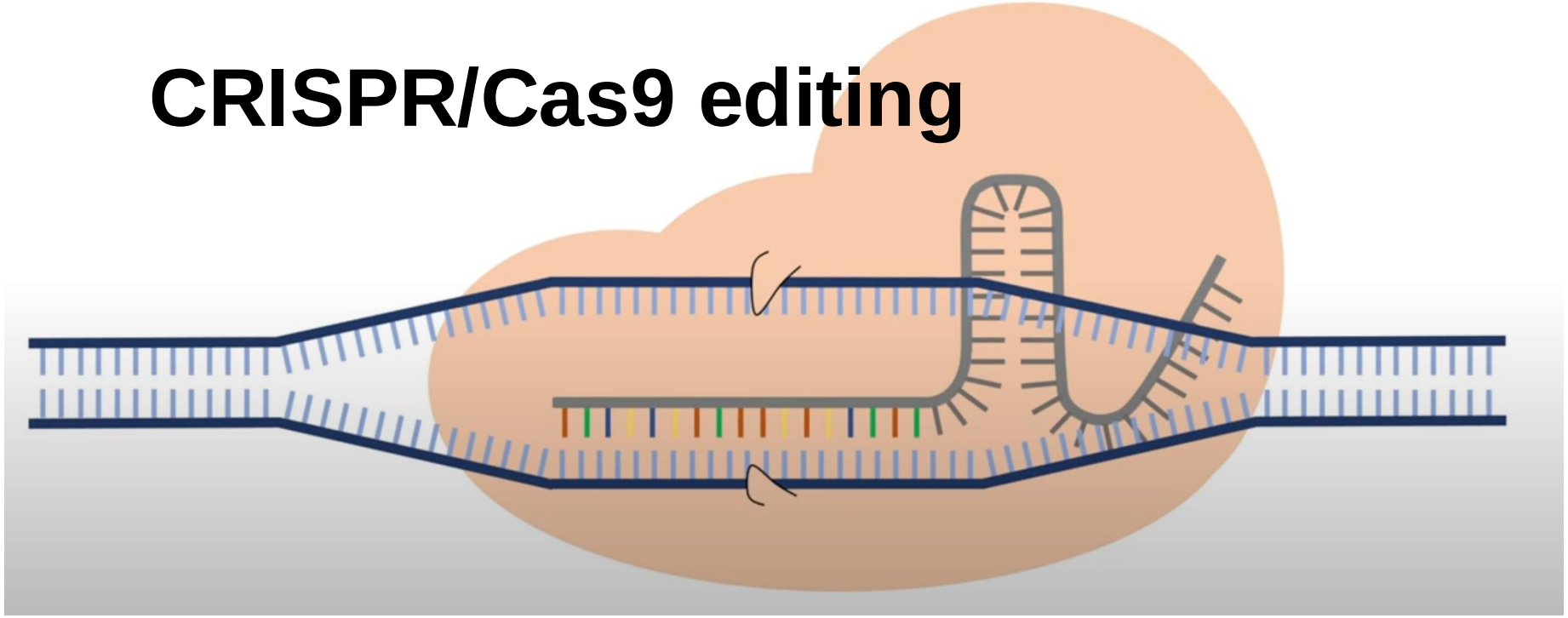
Importance of developing microbiome manipulation strategies



Fecal Microbiota Transplant

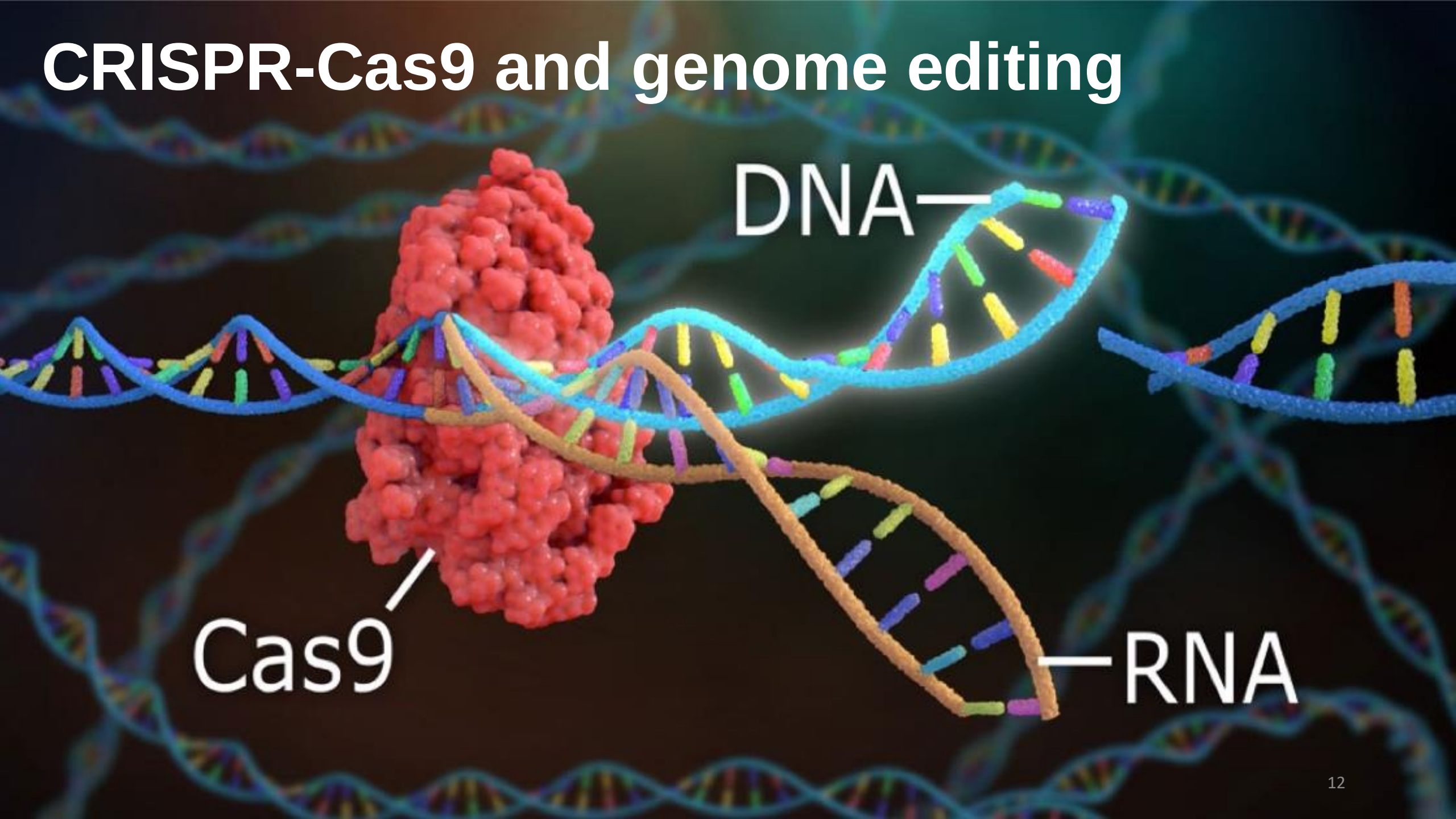
Infection control and antibiotic resistance

A more precise microbiome-editing strategy: CRISPR/Cas9



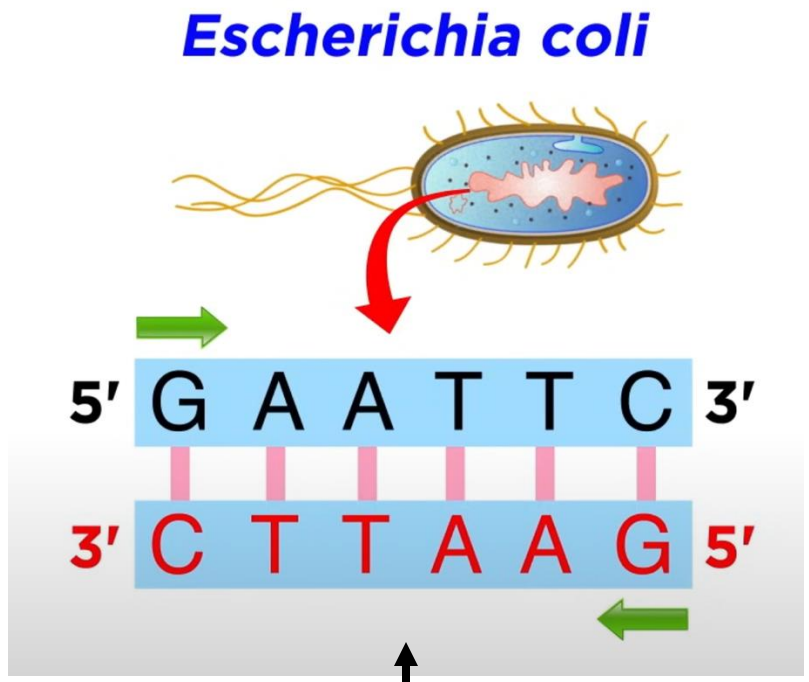
Personalized Medicine

CRISPR-Cas9 and genome editing

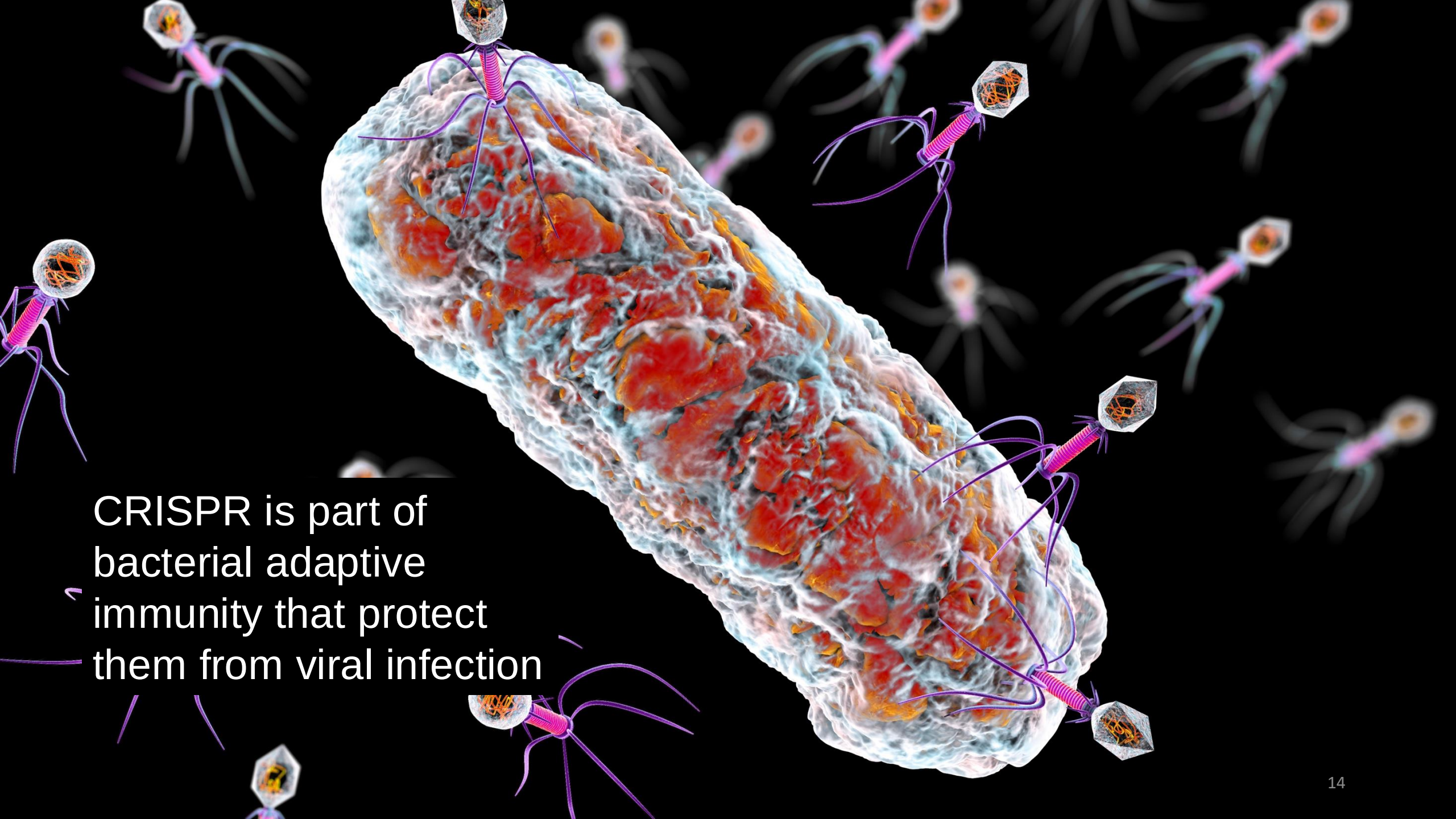


1987: Atsuo Nakata Group Osaka University, Japan

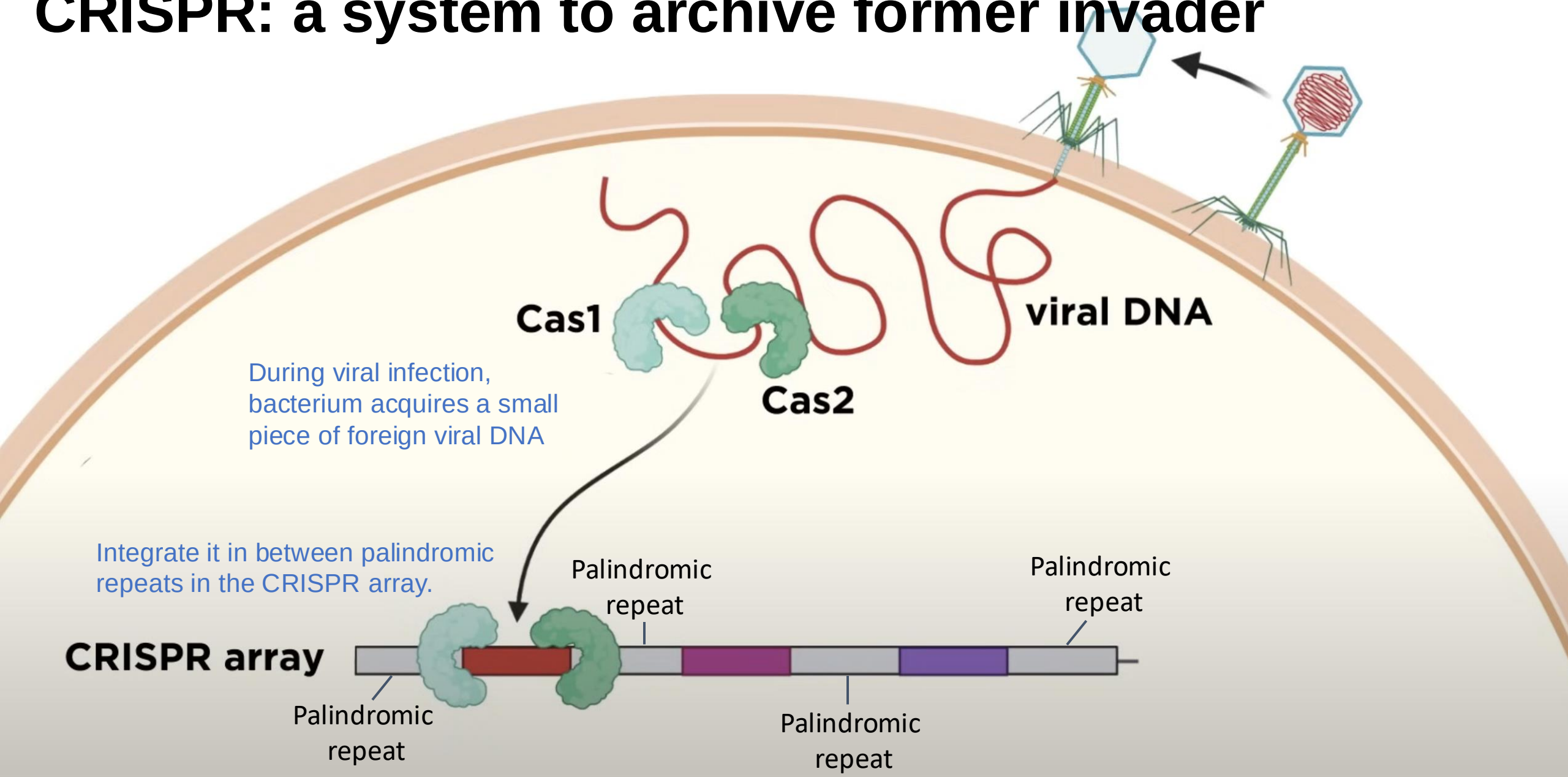
Clustered
Regularly
Interspaced
Short
Palindromic
Repeats



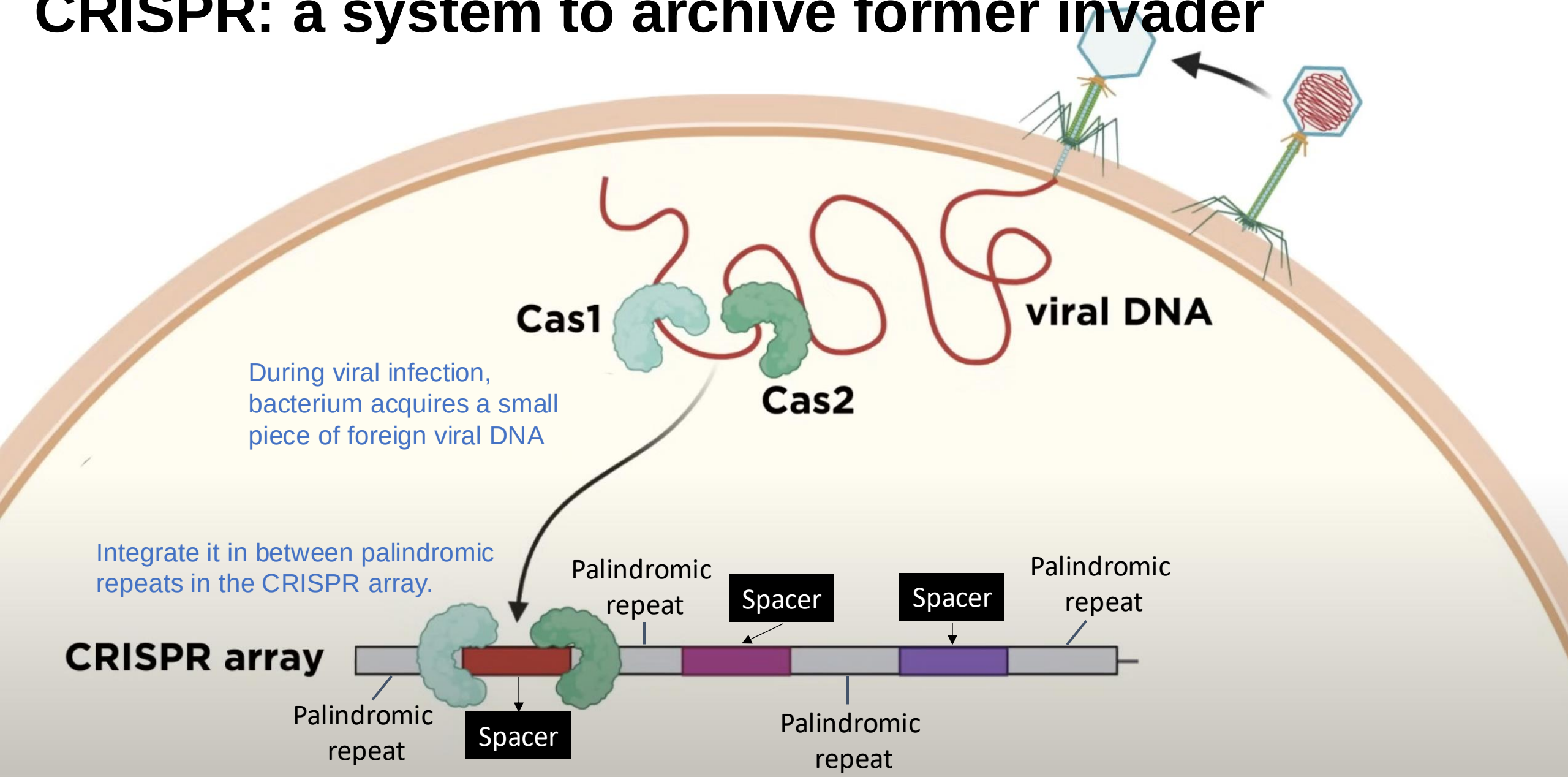
Kayak


CRISPR is part of bacterial adaptive immunity that protect them from viral infection

CRISPR: a system to archive former invader



CRISPR: a system to archive former invader



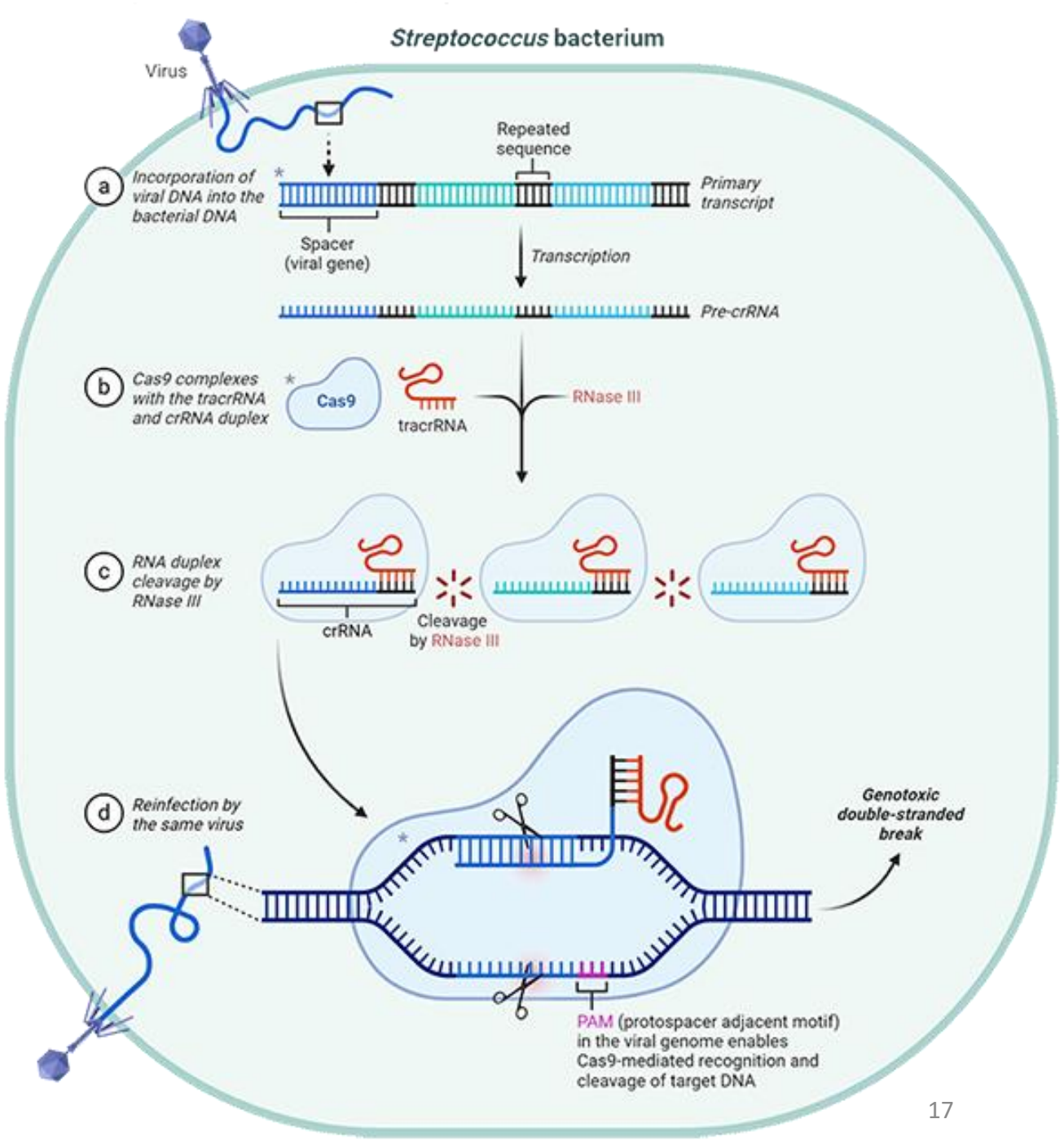
CRISPR defense mechanism in Streptococcus

CRISPR surveillance squad:

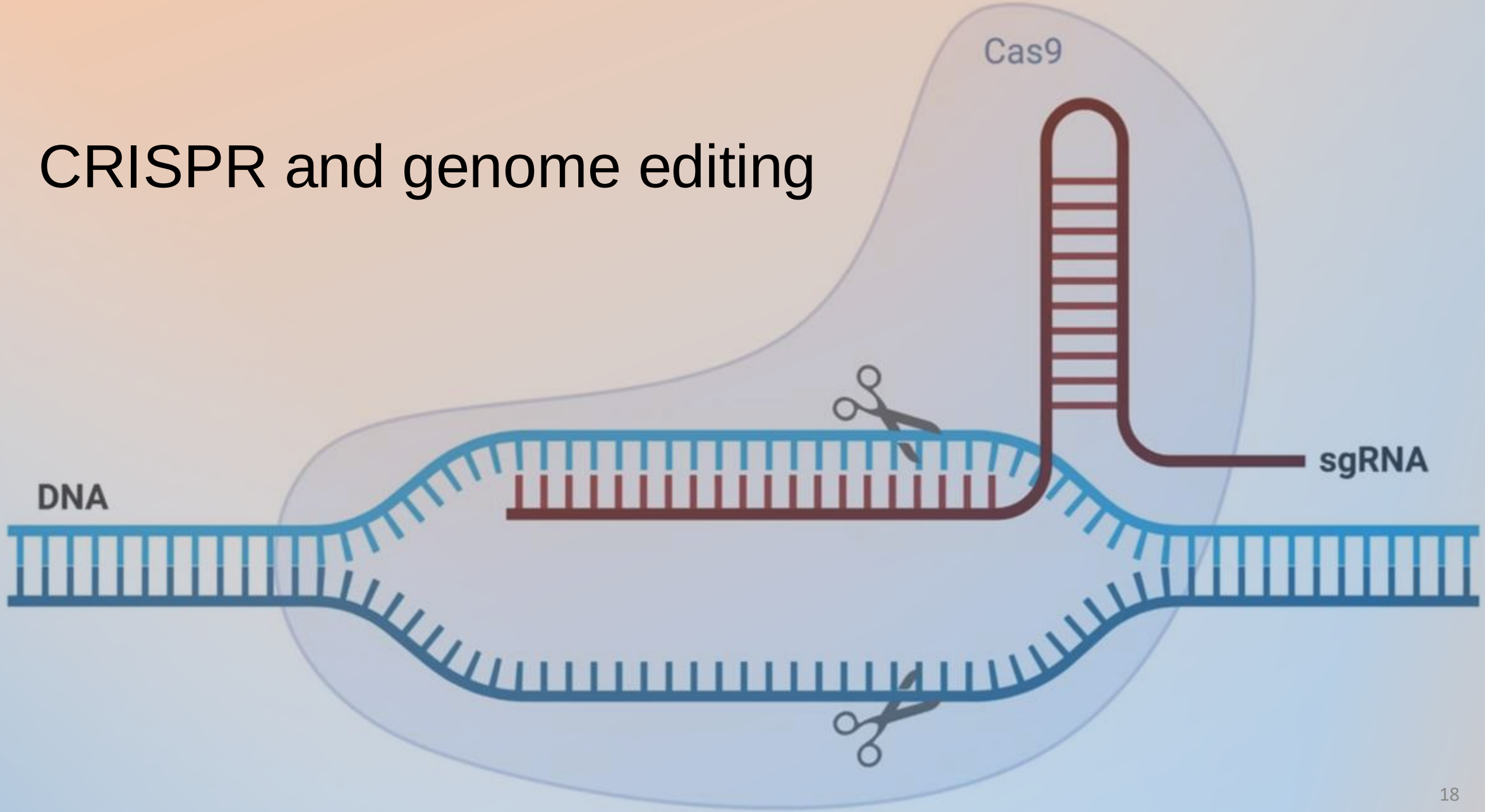
Cas9

tracrRNA

Rnase III

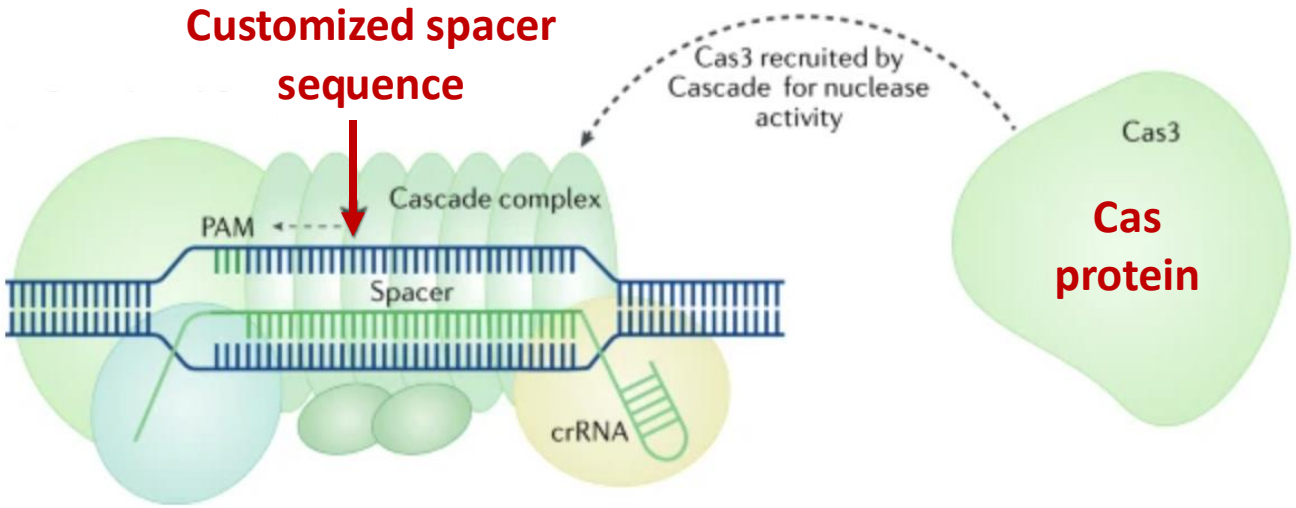


CRISPR and genome editing

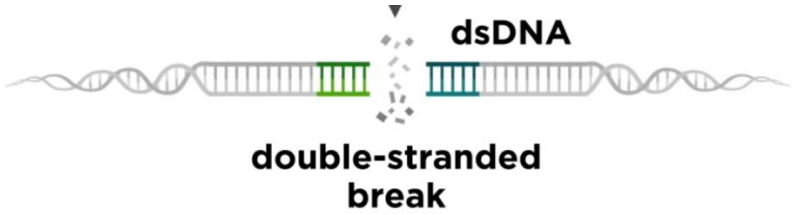


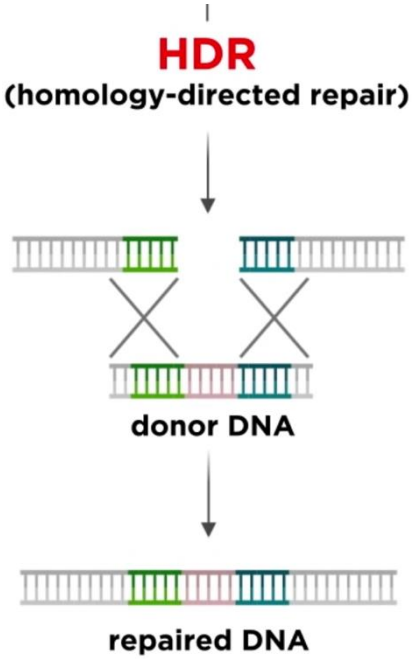
The Nobel Prize In Chemistry 2020

“for the development of a method (CRISPR-Cas9) for genome editing”



Exploit the CRISPR system for gene knockout or knock-in.





Some of the exciting applications of genome editing include:

Basic research

Break DNA sequences encoding cellular parts to learn their functions

Therapeutic genome editing

***In vivo* genome editing**
Treat diseases by delivering genome editing tools directly to the body

***Ex vivo* genome editing**
Edit cells outside the body & later deliver them to patients

Bioproduction

Engineer cells to produce useful compounds

...and much more!

Phage-delivered CRISPR-Cas9 for strain-specific depletion and genomic deletions in the gut microbiome

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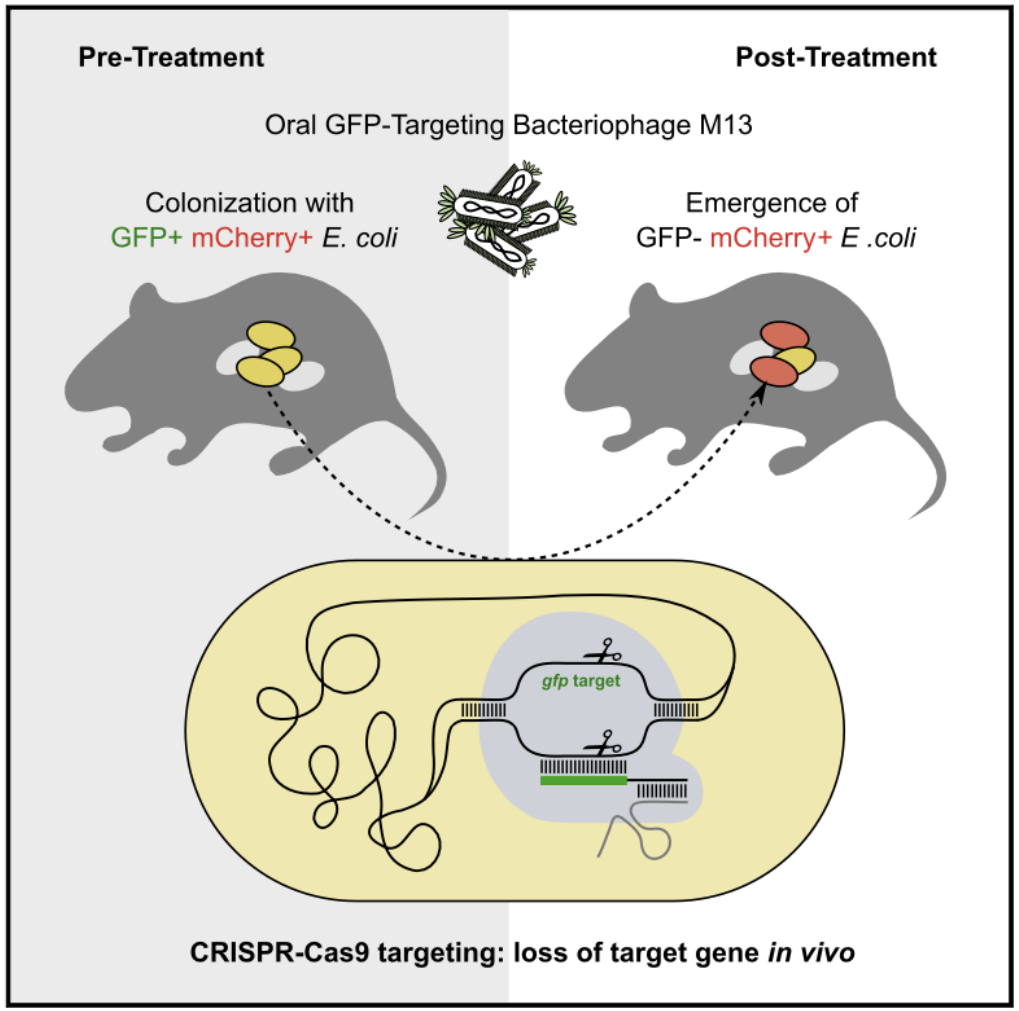

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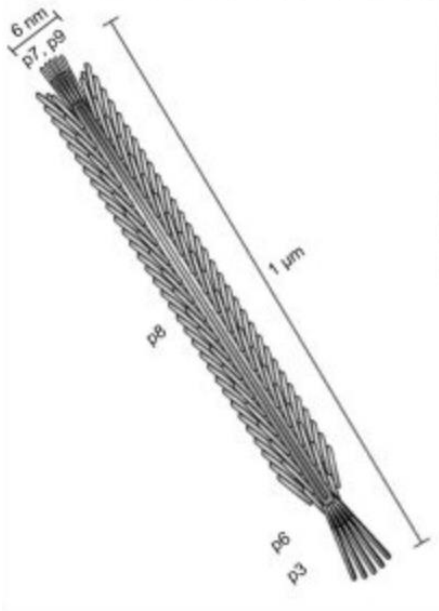
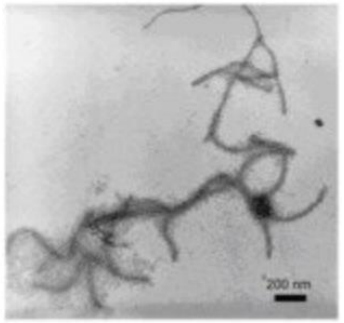
Filamentous bacteriophage M13



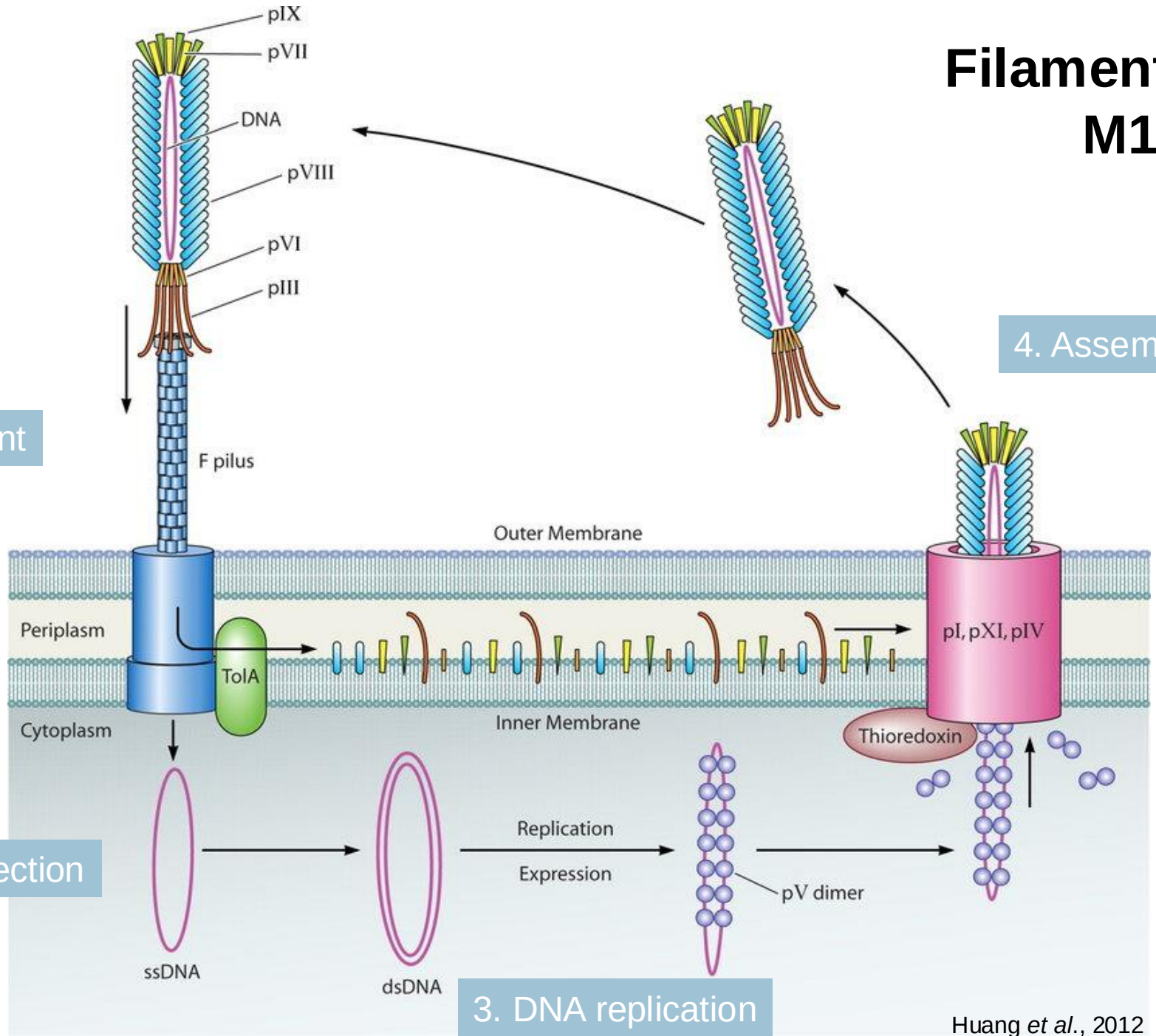


Lee et al., 2013

Single-stranded DNA
(ssDNA) filamentous inovirus

E. coli W1655

Filamentous Phage M13 Life Cycle



1. Attachment

2. DNA injection

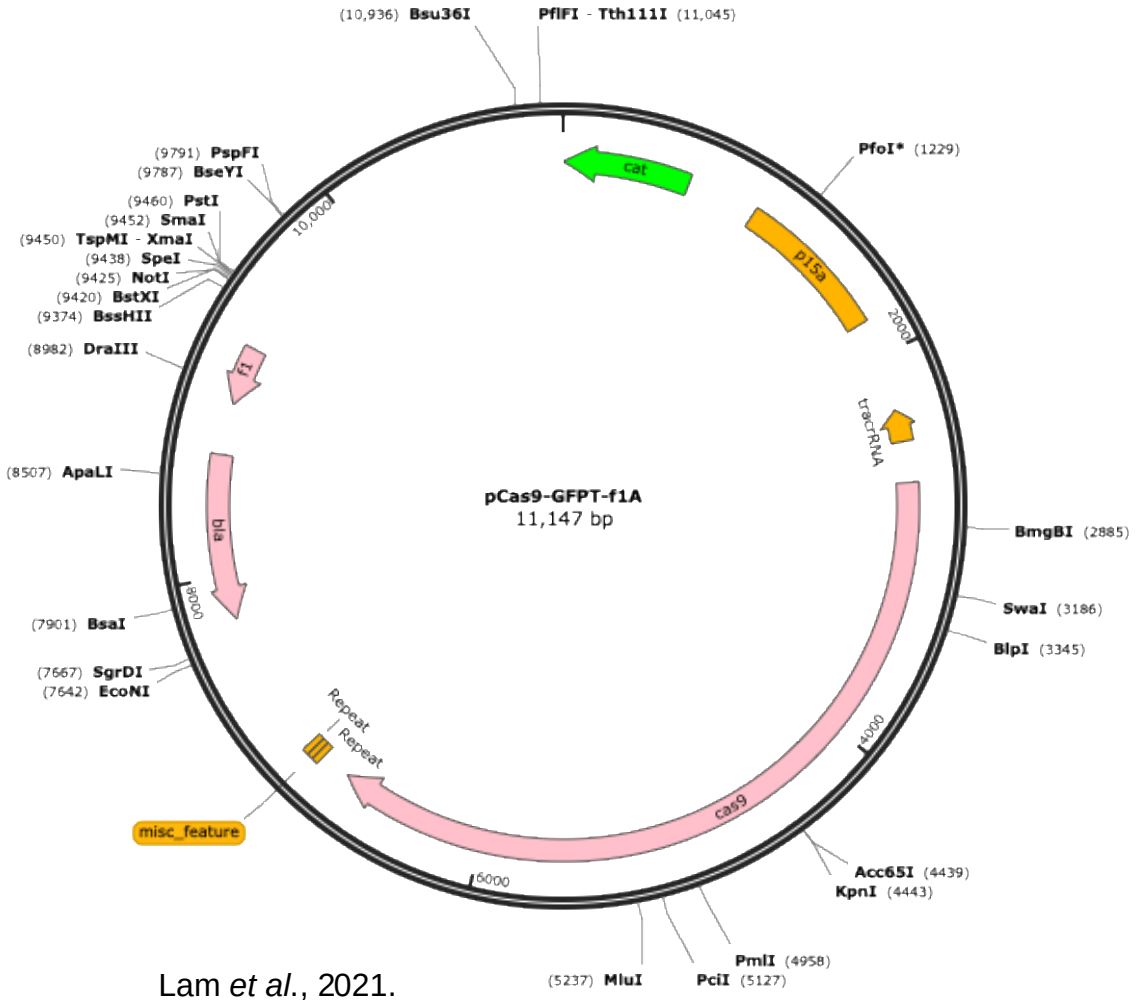
3. DNA replication

4. Assemble and release

Huang *et al.*, 2012

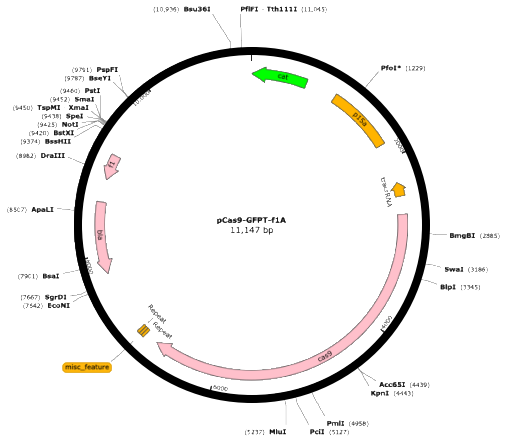
M13 phagemid

The phagemid carries both the CRISPR spacer sequence and the Cas9 protein sequence, which together form the ‘molecular scissors' to cleave the dsDNA in bacteria, achieving specific bacterial strain depletion.



Lam *et al.*, 2021.

Phage-delivered CRISPR-Cas9 system



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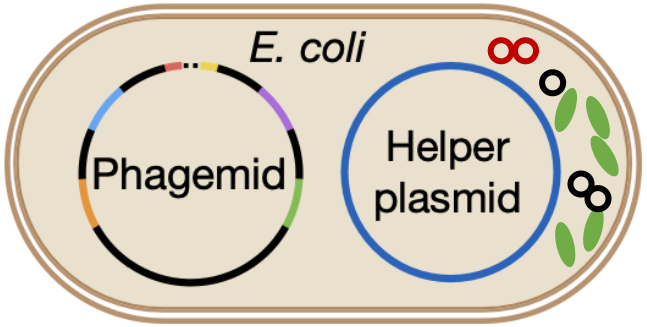
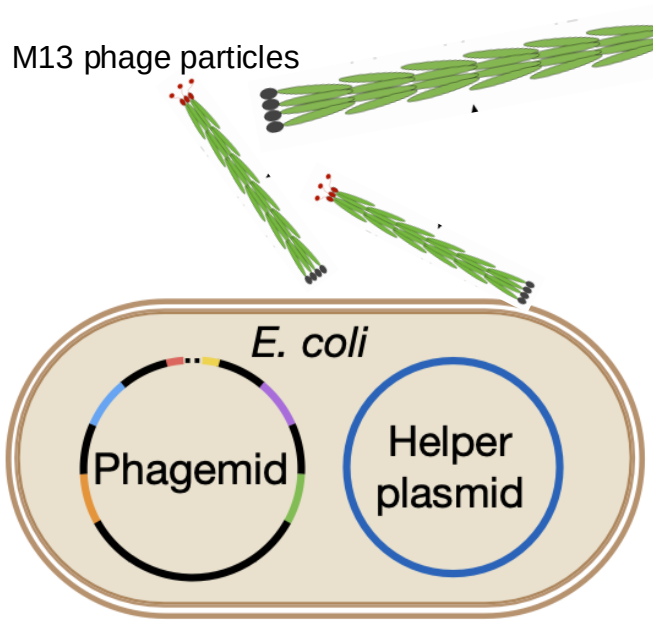


Praetorius *et al.*, 2017.



Phagemid construction	Phagemid transformation	Release of M13 phage particles

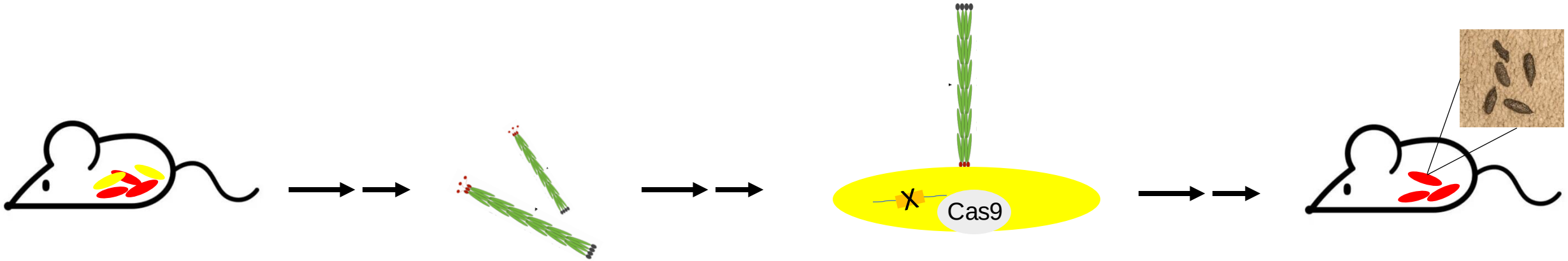
1) Get phagemid into the *E. coli* cell

2) The helper plasmid carries genes encoded for M13 phage.

3) During M13 phage assembly within *E. coli*, phagemid will be packed into the M13 phage.

M13 phage particles carrying CRISPR-Cas9 phagemids are released from *E. coli.*

Animal model: Specific pathogen free (SPF) BALB/c mice



Oral gavage mice with fluorescently labeled *E. coli.*

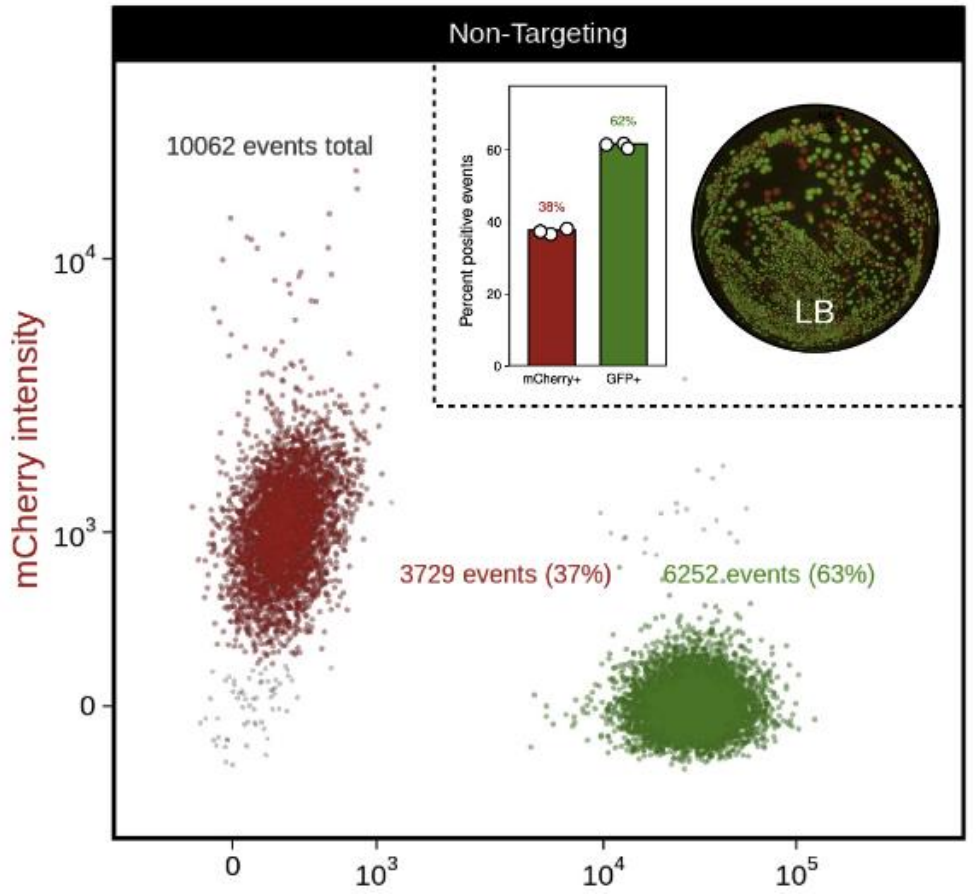
Oral gavage mice with phage M13.

Analyze bacterial strains in mice fecal pellet.

Flow cytometry

BD LSRFortessa flow cytometer

Lam *et al.*, 2021.



- Detect bacterial cells based on fluorescence.
- Quantitative analysis.

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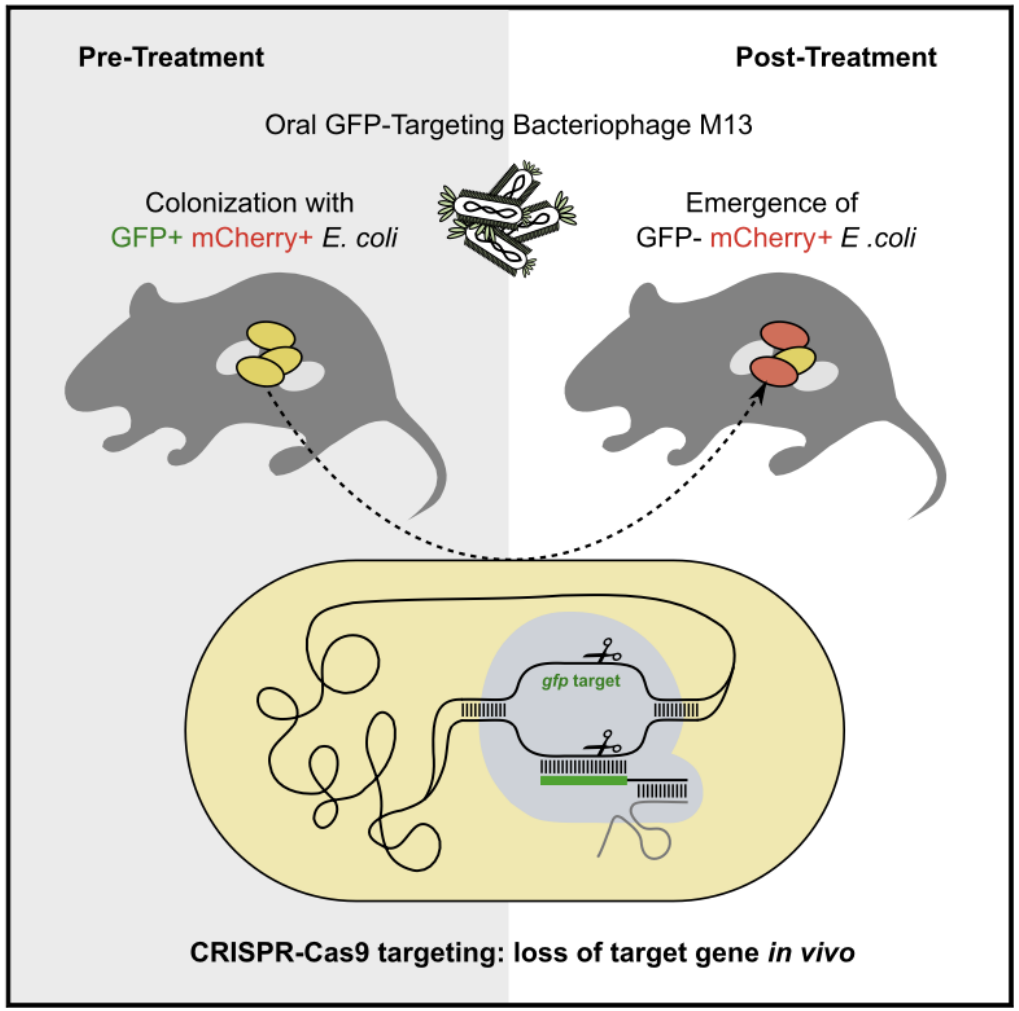

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Q & A