GENOME-WIDE CRISPR SCREENS

Huixian Qiu and Perla Larios

What is CRISPR?

Clustered

Regularly

Interspaced

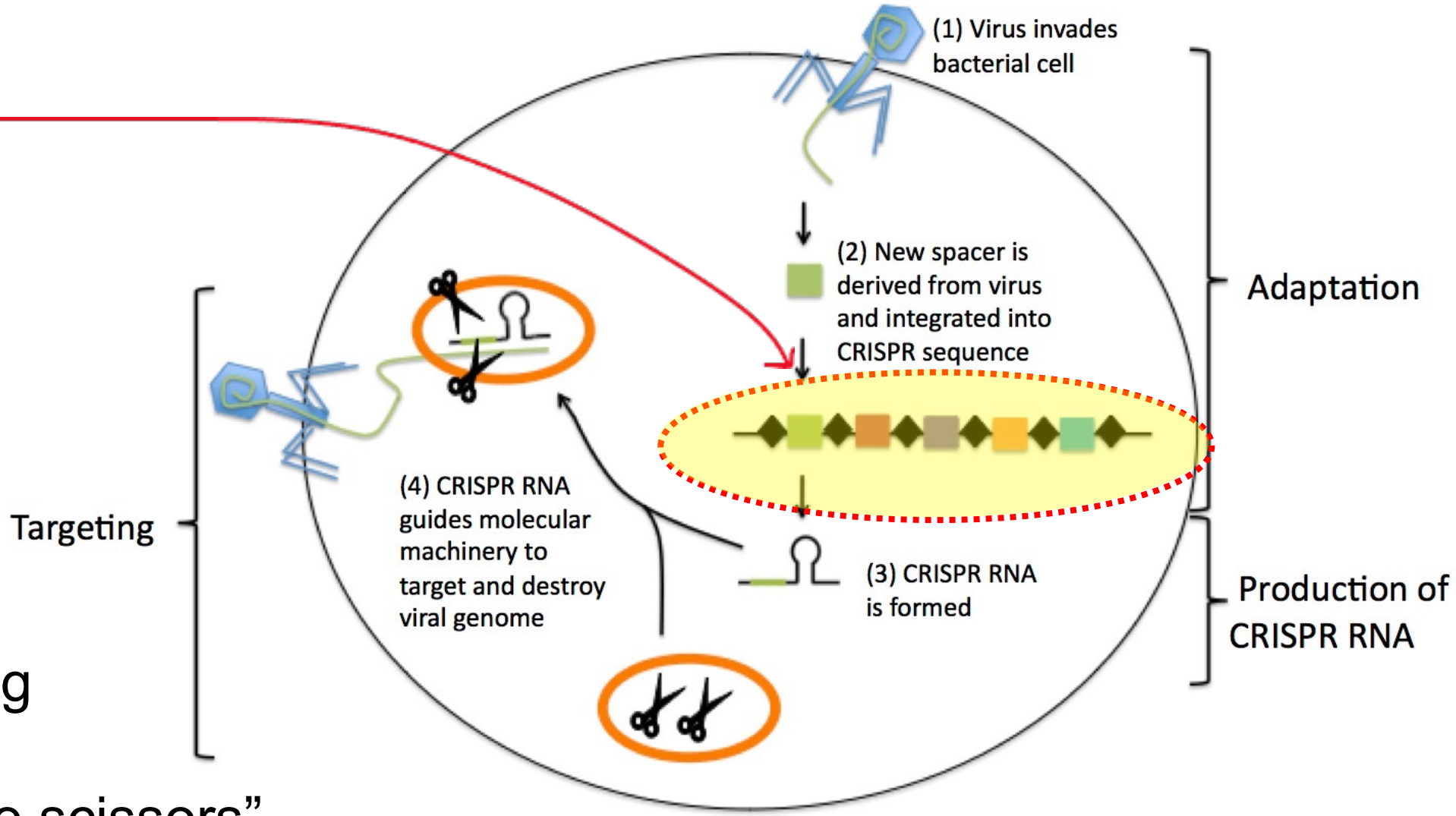
Short

Palindromic

Repeats

Genome editing

“Programmable scissors”



Barrangou, R. and Marraffini, L. CRISPR-Cas Systems: Prokaryotes Upgrade to Adaptive Immunity (2014). Molecular Cell 54, 234-244.

How was CRISPR first discovered?

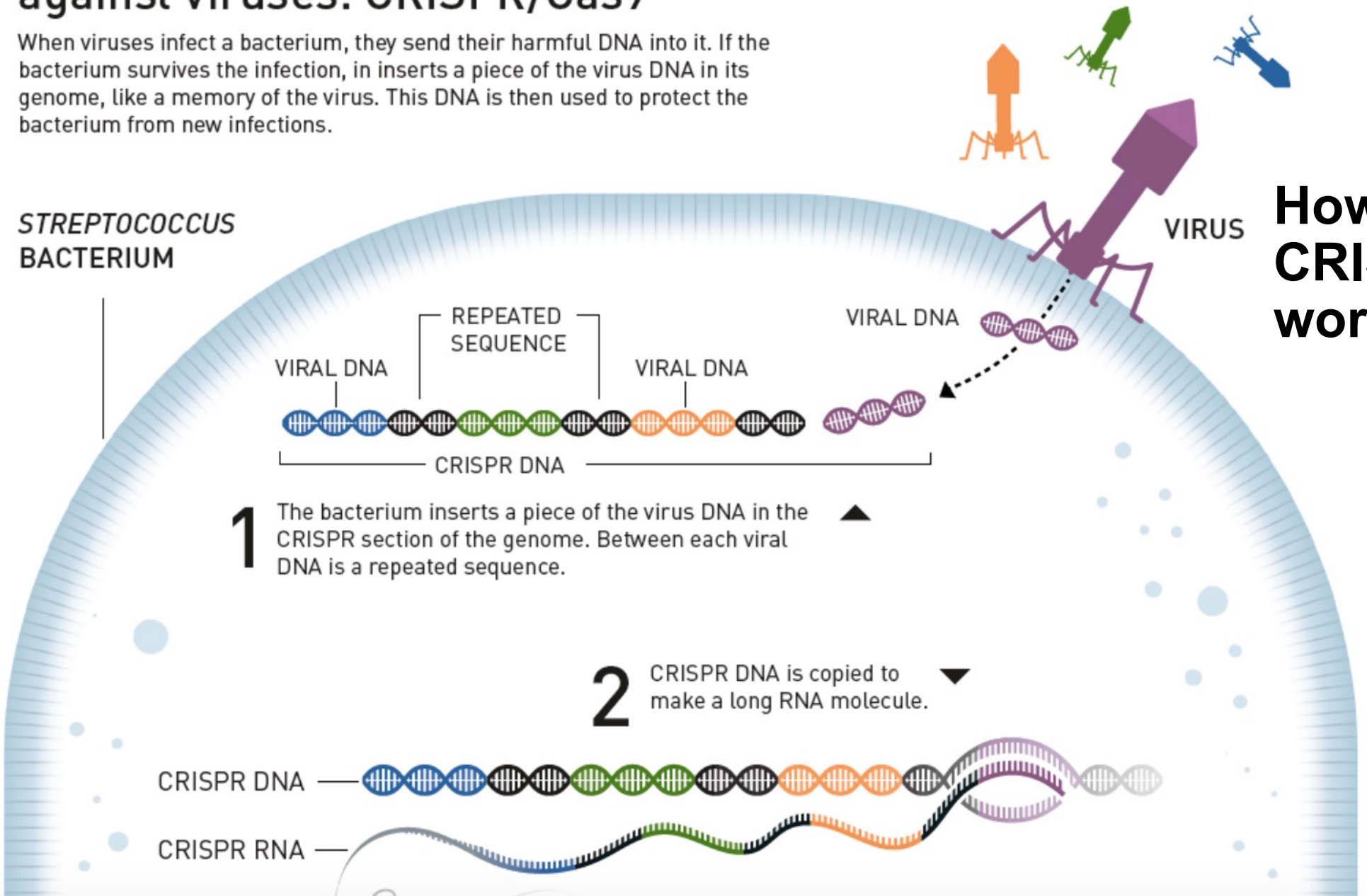
Reported in 1993 by **Dr. Francisco Mojica** University of Alicante, Spain (also coined the term)

First discovered in **archaea** (and later in bacteria)

Hypothesis: CRISPR is an **adaptive immune system**

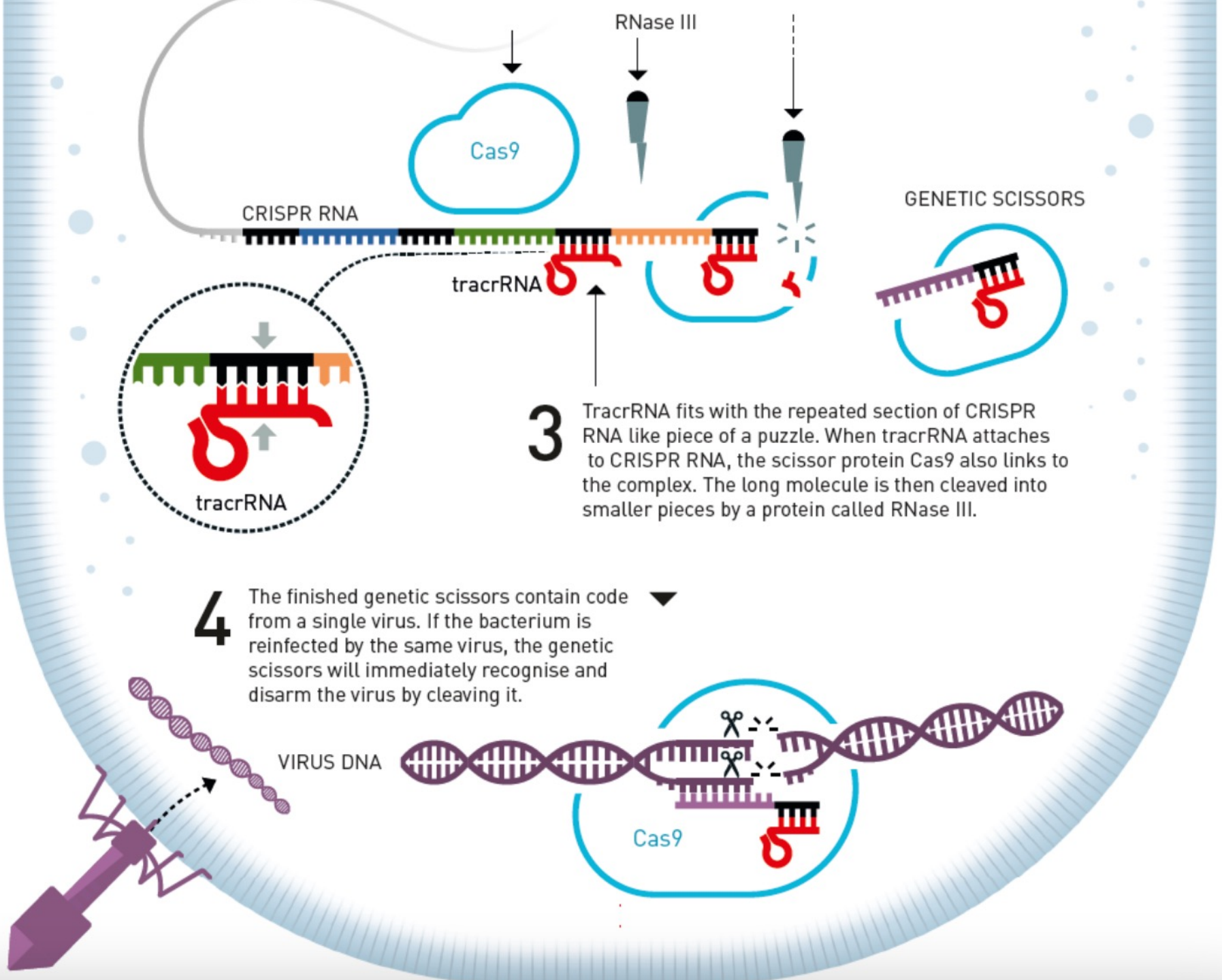
Streptococcus' natural immune system against viruses: CRISPR/Cas9

When viruses infect a bacterium, they send their harmful DNA into it. If the bacterium survives the infection, in inserts a piece of the virus DNA in its genome, like a memory of the virus. This DNA is then used to protect the bacterium from new infections.



How does CRISPR system work in bacteria?

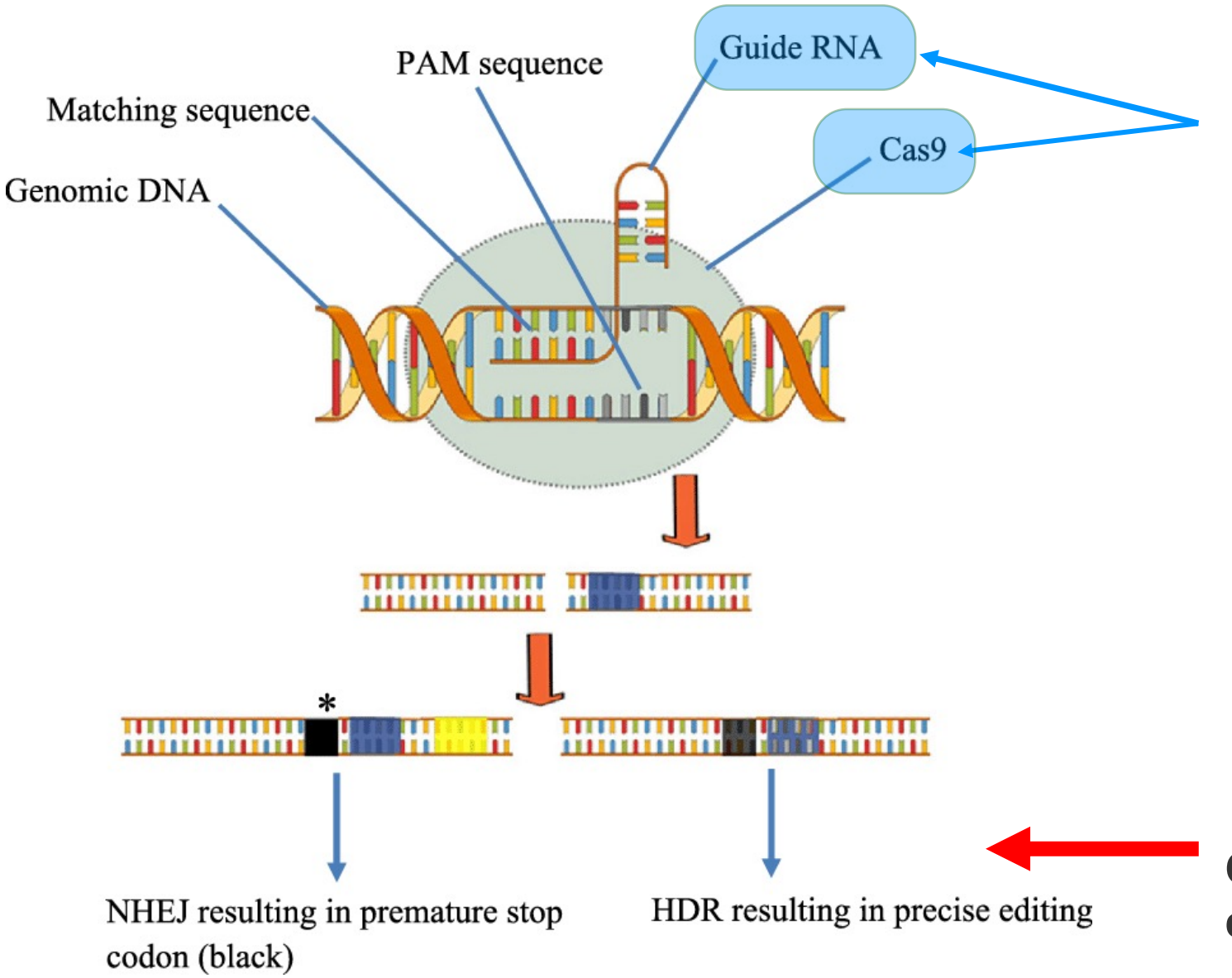
Part 2: How does CRISPR system work in bacteria?



Nobel Prize in Chemistry 2020: Emmanuelle Charpentier and Jennifer Doudna

Who developed CRISPR-Cas9 system?

How does CRISPR work?



Key Components

2 repair pathways for CRISPR mediated double stranded breaks

CRISPR/Cas9: the Jedi against the darkempire of diseases (2018) Sehrish Khan Et al.

How to compare different gene editing methods?

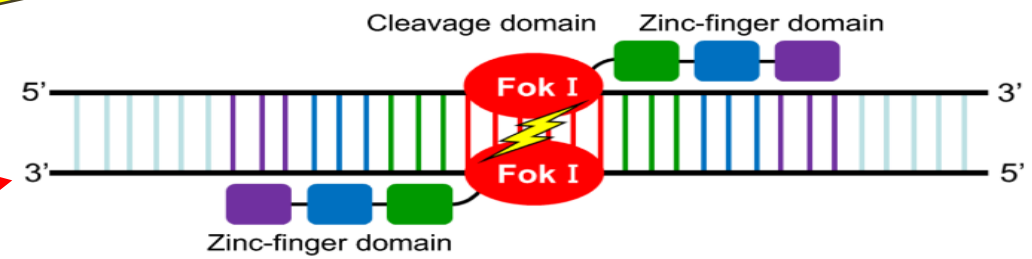
RNAi → RNA-basd, KD

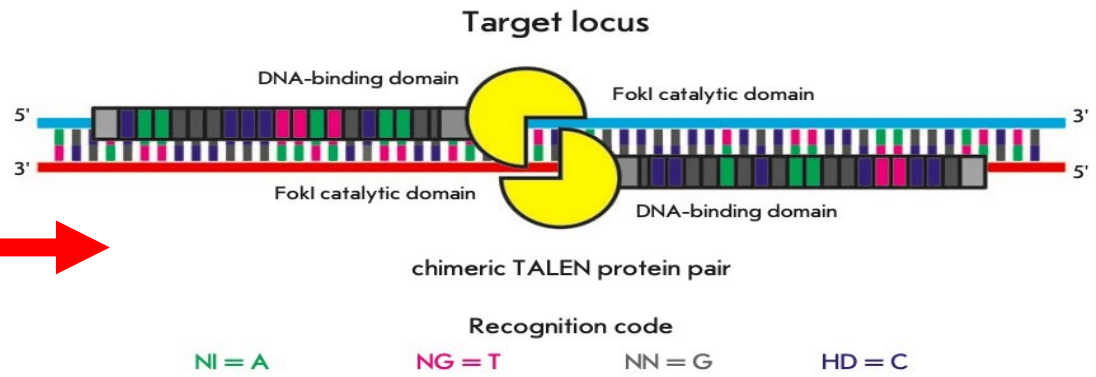
CRISPR ★

Zinc-Finger Nucleases (ZFNs)

Transcription Activator–Like Effector Nucleases (TALENs)

DNA-based,KO

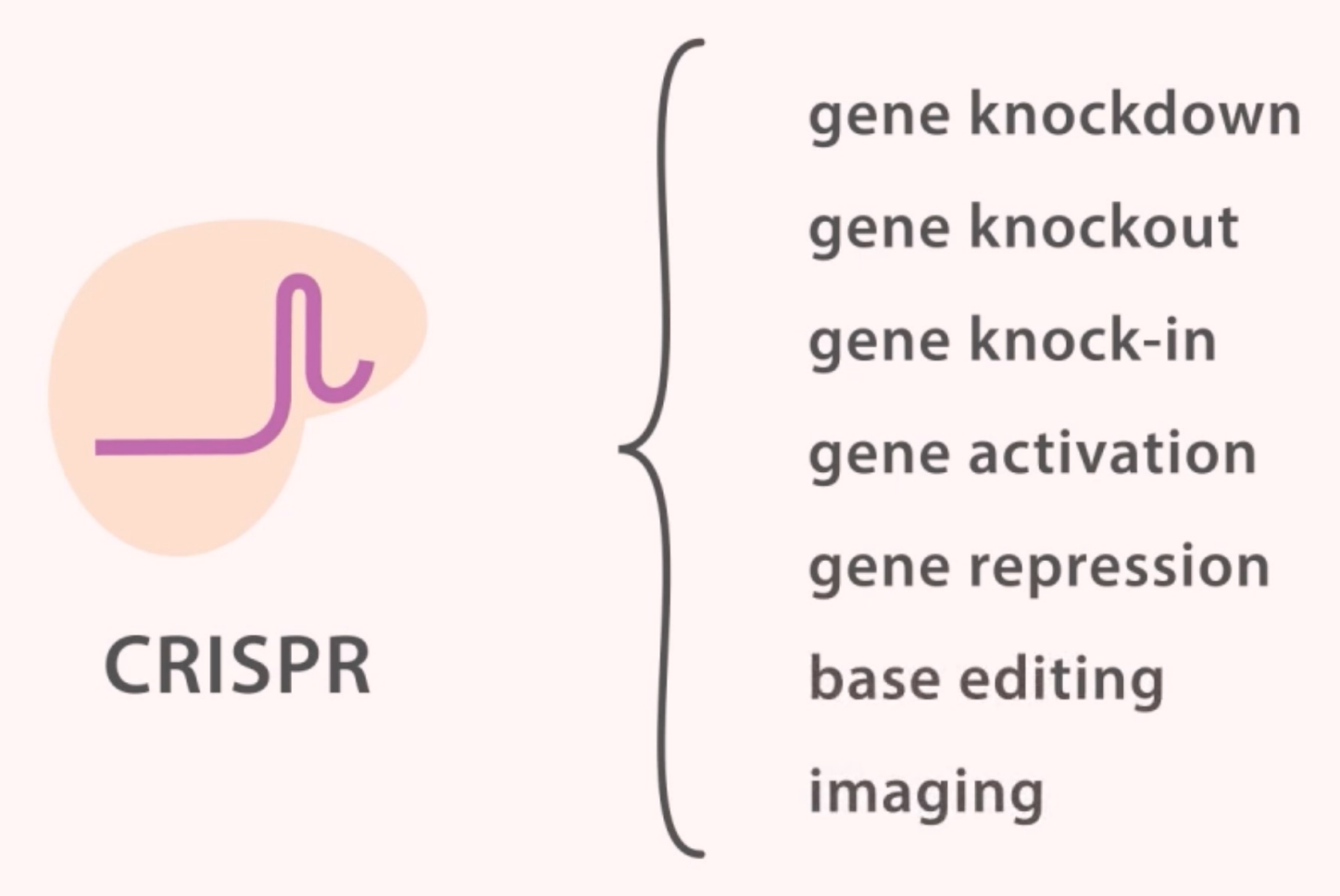




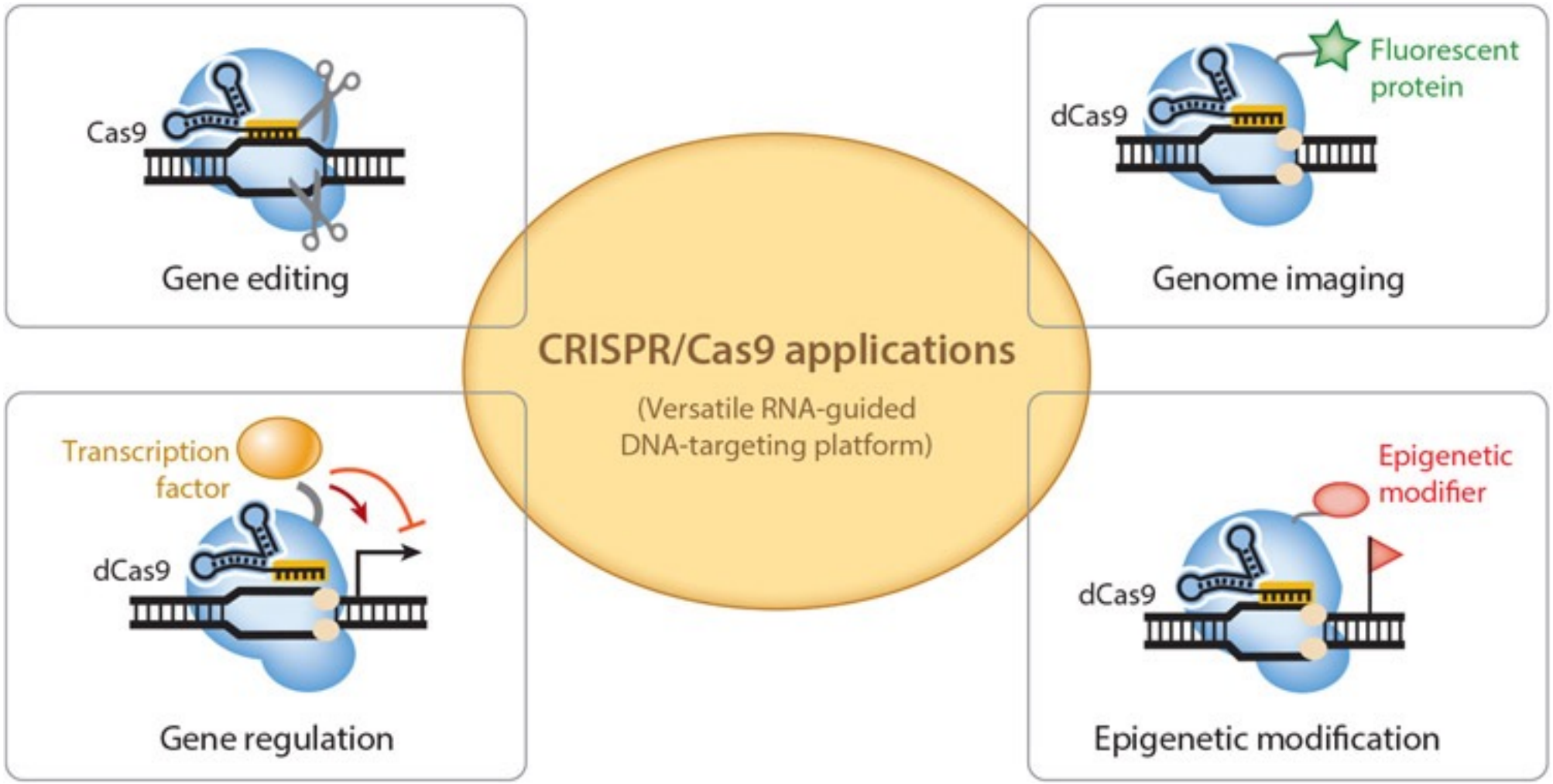
Boettcher M, McManus MT. Choosing the Right Tool for the Job: RNAi, TALEN, or CRISPR. Mol Cell. 2015 May 21;58(4):575-85. doi: 10.1016/j.molcel.2015.04.028. PMID: 26000843; PMCID: PMC4441801.

Why is CRISPR so powerful and important?

- Simplicity
- Precision
- Versatility

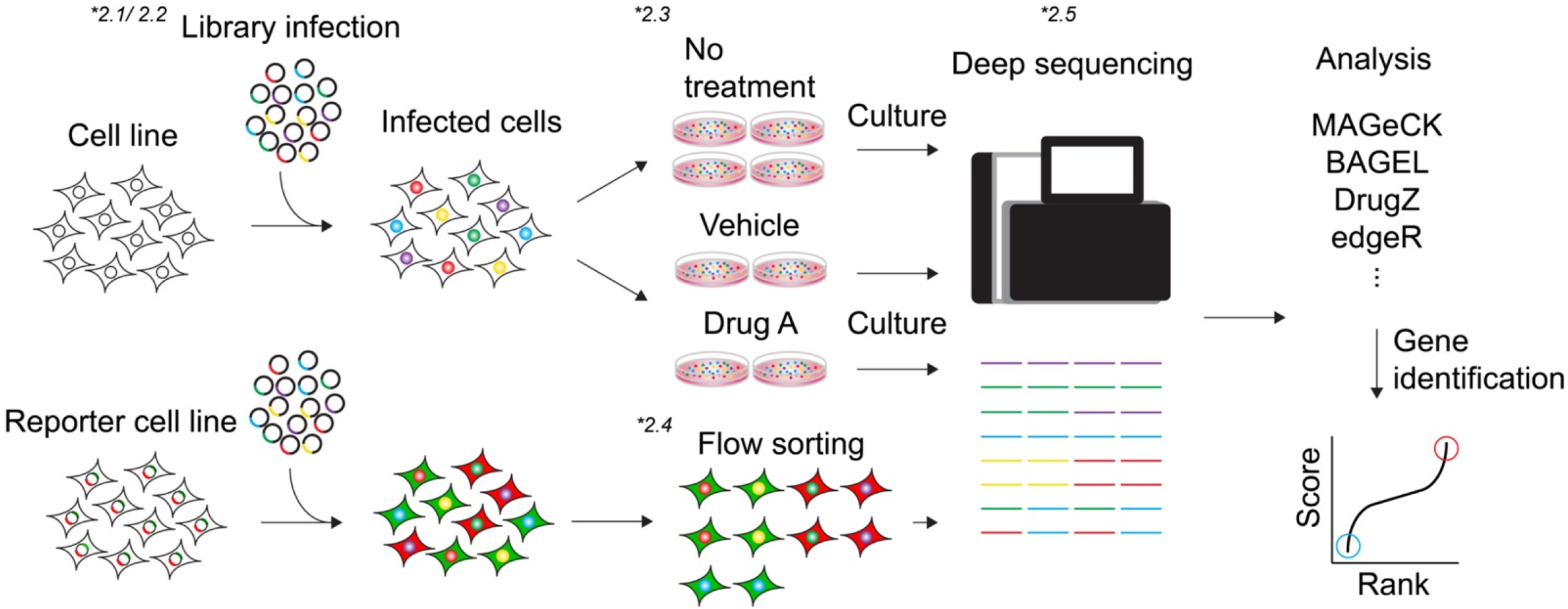


What can CRISPR/Cas9 system do?



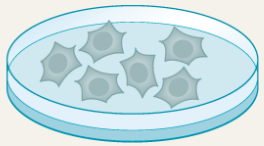
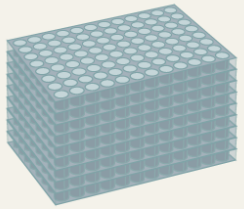
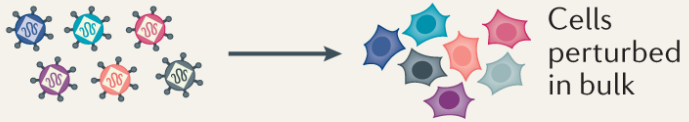
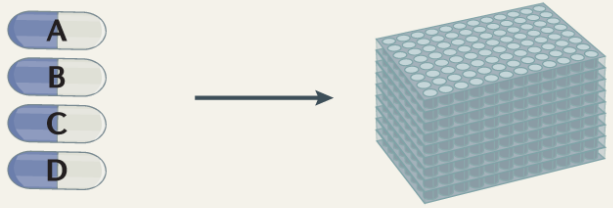
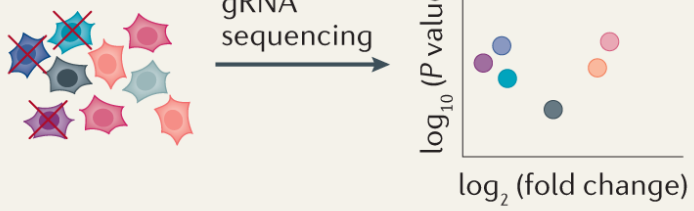
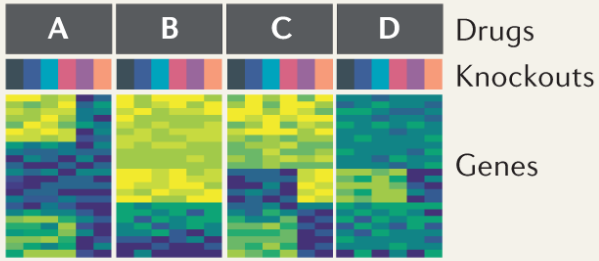
Today's focus: CRISPR loss-of-function **screens**

How can CRISPR be used to perform a genetic screen?

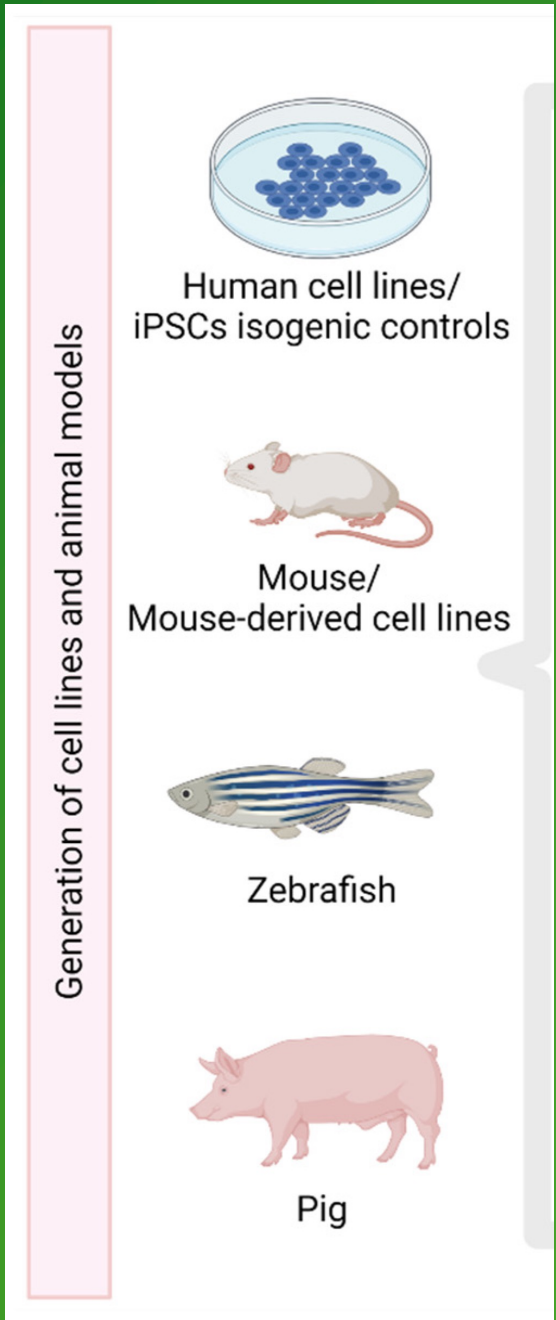


https://www.mdpi.com/1422-0067/22/23/12777

What are different types of CRISPR screening?

	Pooled CRISPR screen	Arrayed CRISPR screen
Model Cell lines, primary cells, organoids, model organisms (in vivo screens)	Cas9-expressing cells in bulk 	Cas9-expressing cells in separate wells 
Perturbation CRISPR knockout, interference, activation, base editing, prime editing	CRISPR gRNA library  Cells perturbed in bulk	Cells perturbed in separate wells 
Challenge Cell survival and proliferation, drug treatment and resistance, virus/pathogen infection, metabolic challenges	 Cells challenged in bulk (for example with a drug)	Cells challenged in separate wells (for example with a drug) 
Read-out Sequencing-based counting of gRNA frequencies, single-cell RNA sequencing, multi-omics profiling, imaging		Molecular phenotyping 

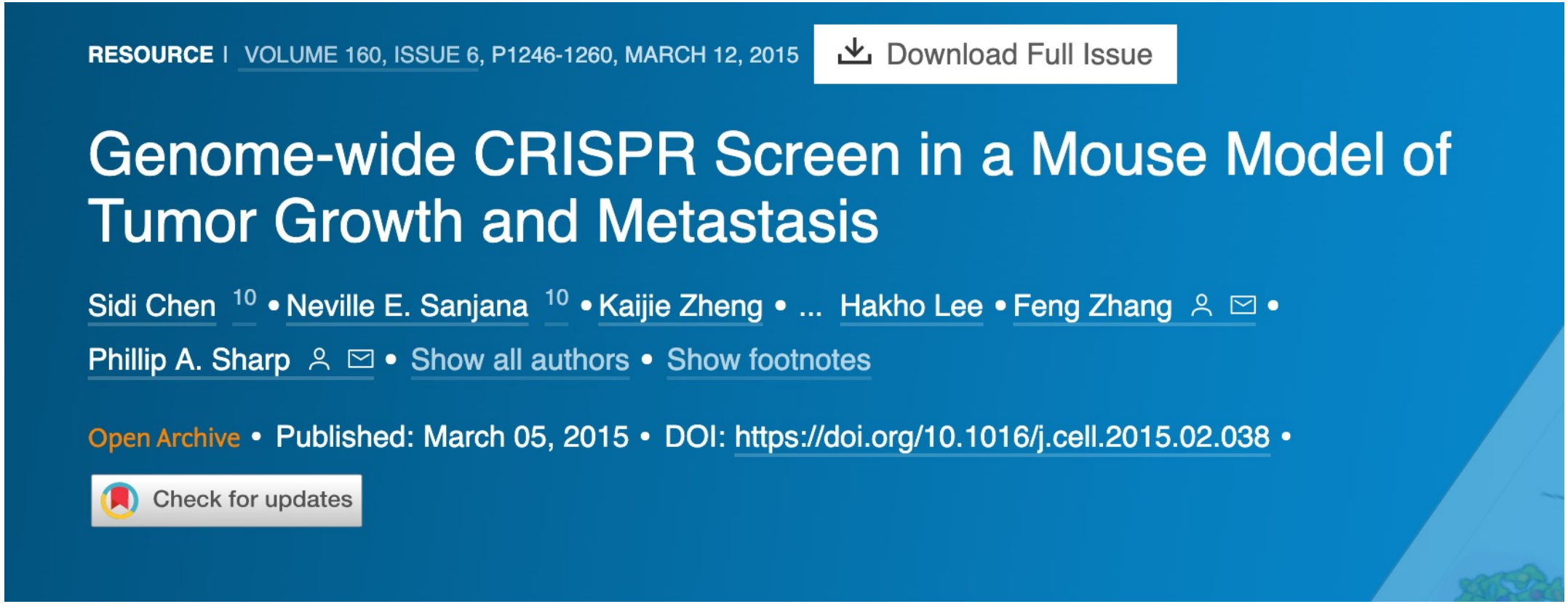
Bock, C., Datlinger, P., Chardon, F. *et al.* High-content CRISPR screening. *Nat Rev Methods Primers* **2**, 8 (2022). https://doi.org/10.1038/s43586-021-00093-4



What organisms can you use to perform CRISPR screens?

- Human cell lines / iPSCs isogenic controls
- Mouse/ Mouse-derived cell lines
- Zebrafish
- Pig
- Yeast
- And more...

What is an example of CRISPR screen in a model organism?

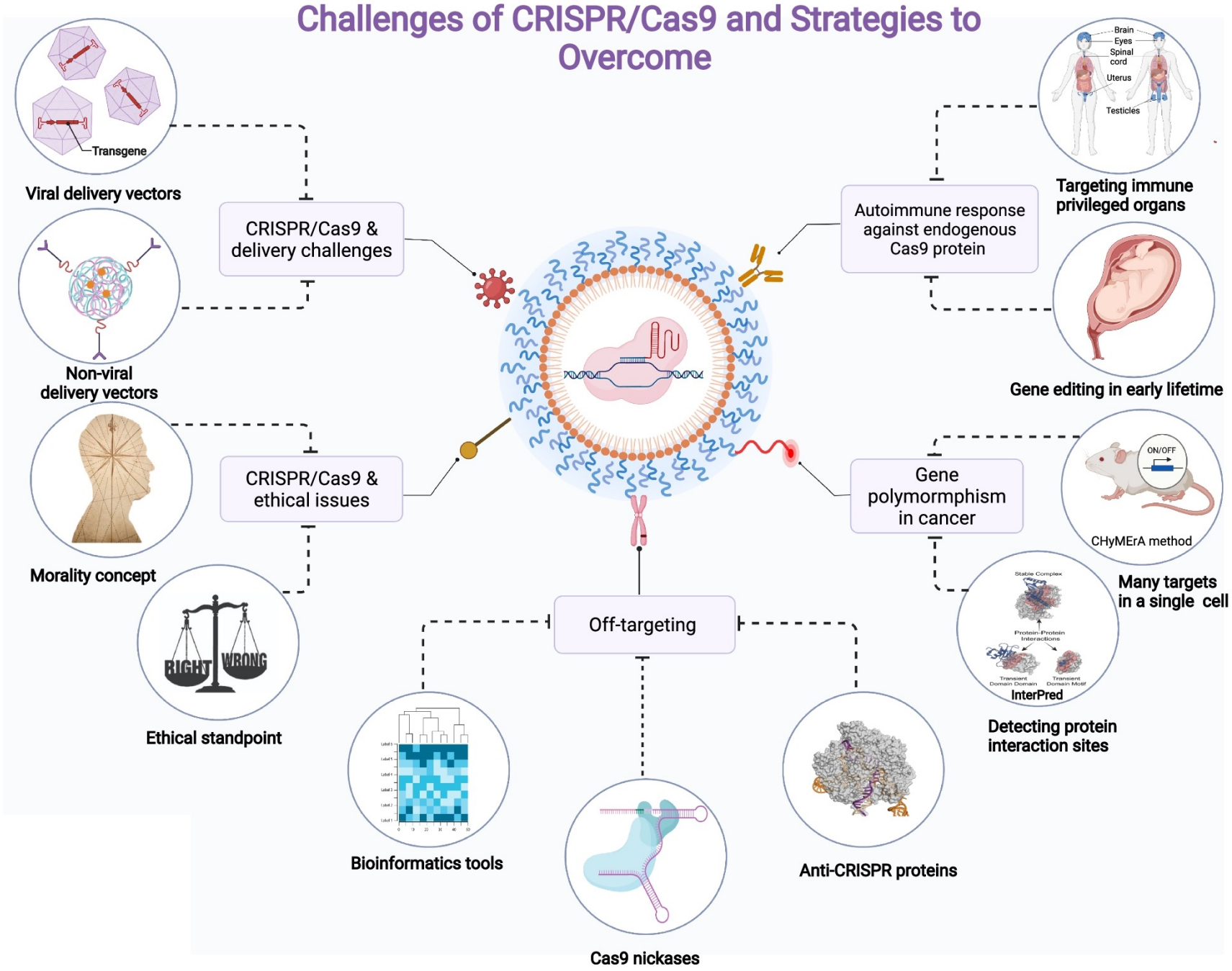


Concerns about CRISPR?

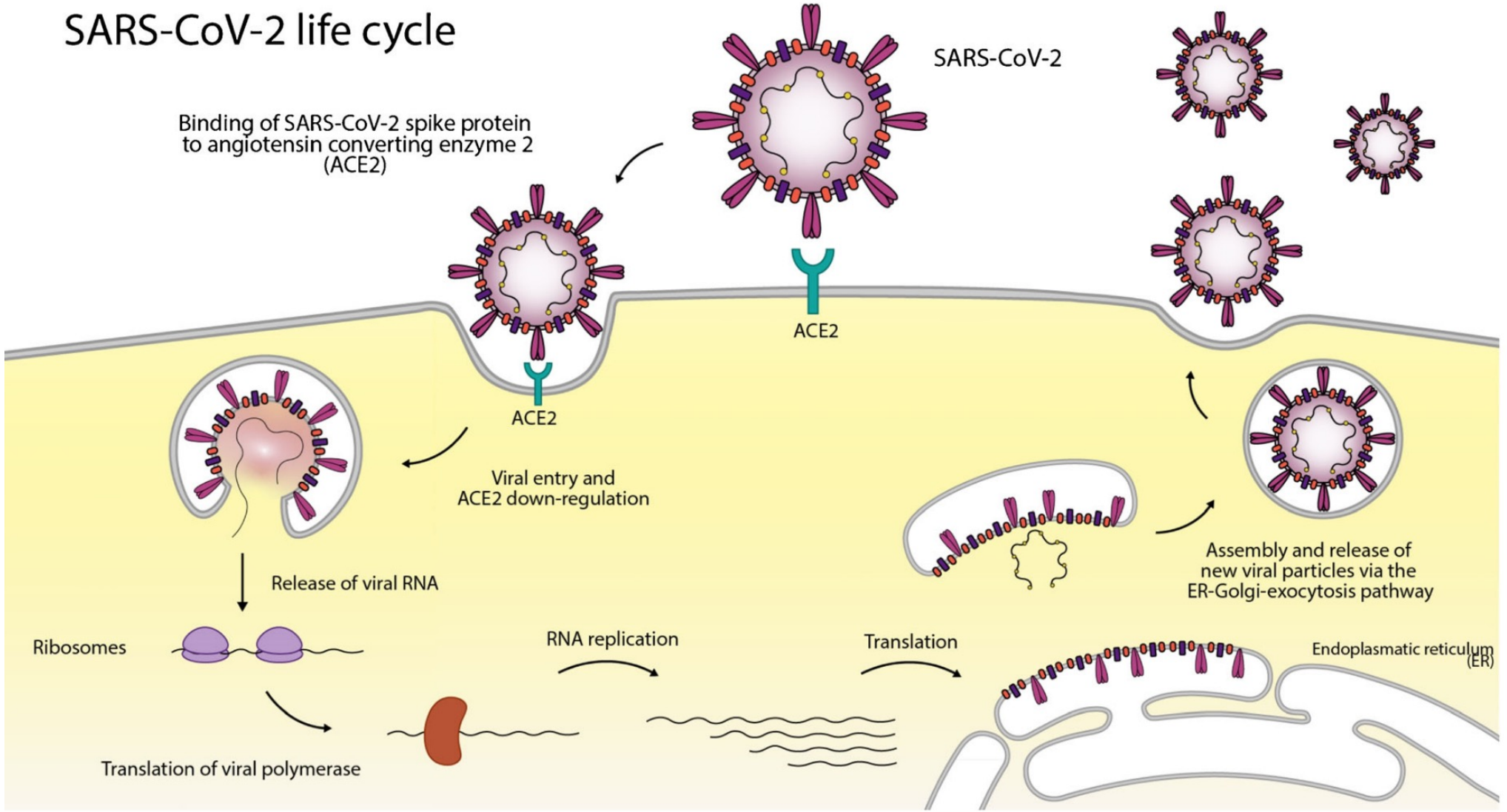
- He Jiankui affair

(Lulu and Nana Controversy)

What are the challenges of CRISPR?



SARS-CoV-2 Life Cycle – How does infection occur?



COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection Stephany Beyerstedt1 & Expedito Barbosa Casaro1 & Érika Bevilaqua Rangel1,2

What's the knowledge gap in COVID-19?

Lack of genome-wide studies that directly identify human genes required for viral infection

Meet the Scientists:

The Sanjana Lab

Dr. Zharko Daniloski

The first author

Dr. Neville Sanjana

(PI)

Affiliation: NY Genome Center and NYU

Lab Focus: bioengineering, neuroscience and cancer

The TenOever Lab

Dr. Tristan X. Jordan

The first author

Dr. Benjamin TenOever

(PI)

Affiliation: The Alexandria Center for Life Science – NYC

Lab Focus: Virology, Synthetic Biology, Evolution

Summary

CRISPR-Cas9 system is a powerful genome editing tool that allow us to precisely alters DNA sequence

Genome-wide CRISPR screens target knock-out of genes in the human genome and is used to investigate the roles of genes

Different model organisms can be used depending on the purposes

Thank you!

Reference:

Boettcher M, McManus MT. Choosing the Right Tool for the Job: RNAi, TALEN, or CRISPR. Mol Cell. 2015 May 21;58(4):575-85. doi: 10.1016/j.molcel.2015.04.028. PMID: 26000843; PMCID: PMC4441801.

Daniloski Z, Jordan TX, Wessels HH, Hoagland DA, Kasela S, Legut M, Maniatis S, Mimitou EP, Lu L, Geller E, Danziger O, Rosenberg BR, Phatnani H, Smibert P, Lappalainen T, tenOever BR, Sanjana NE. Identification of Required Host Factors for SARS-CoV-2 Infection in Human Cells. Cell. 2021 Jan 7;184(1):92-105.e16. doi: 10.1016/j.cell.2020.10.030. Epub 2020 Oct 24. PMID: 33147445; PMCID: PMC7584921.

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https://www.youtube.com/watch?v=U3Z4u0DKbx0&ab_channel=AppliedBiologicalMaterials-abm

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https://www.idtdna.com/pages/education/decoded/article/overview-what-is-crispr-screening

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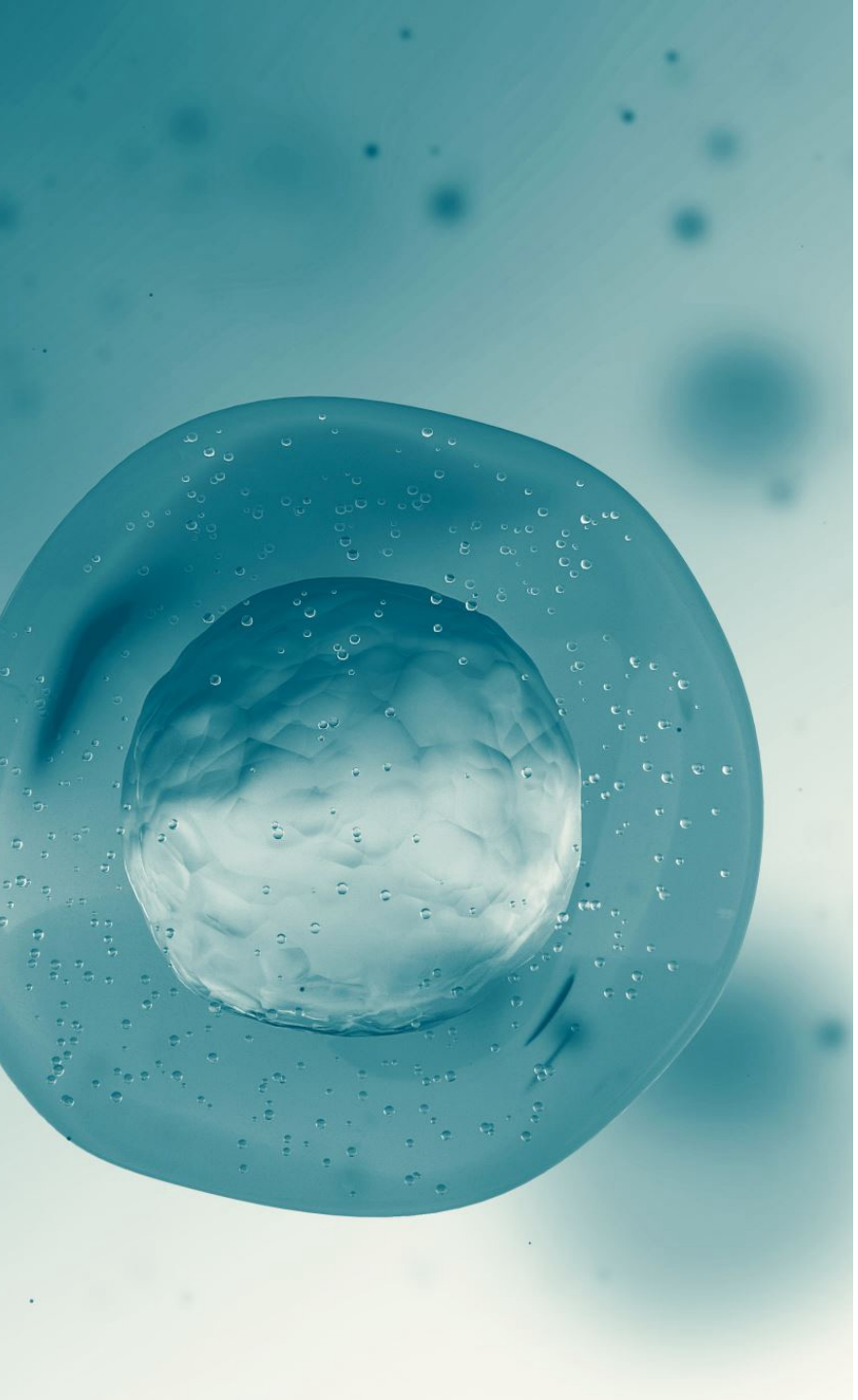


Identification of Required Host Factors for SARS-CoV-2 Infection in Human Cells

Perla Larios and Huixian Qiu

University of Madison- Wisconsin

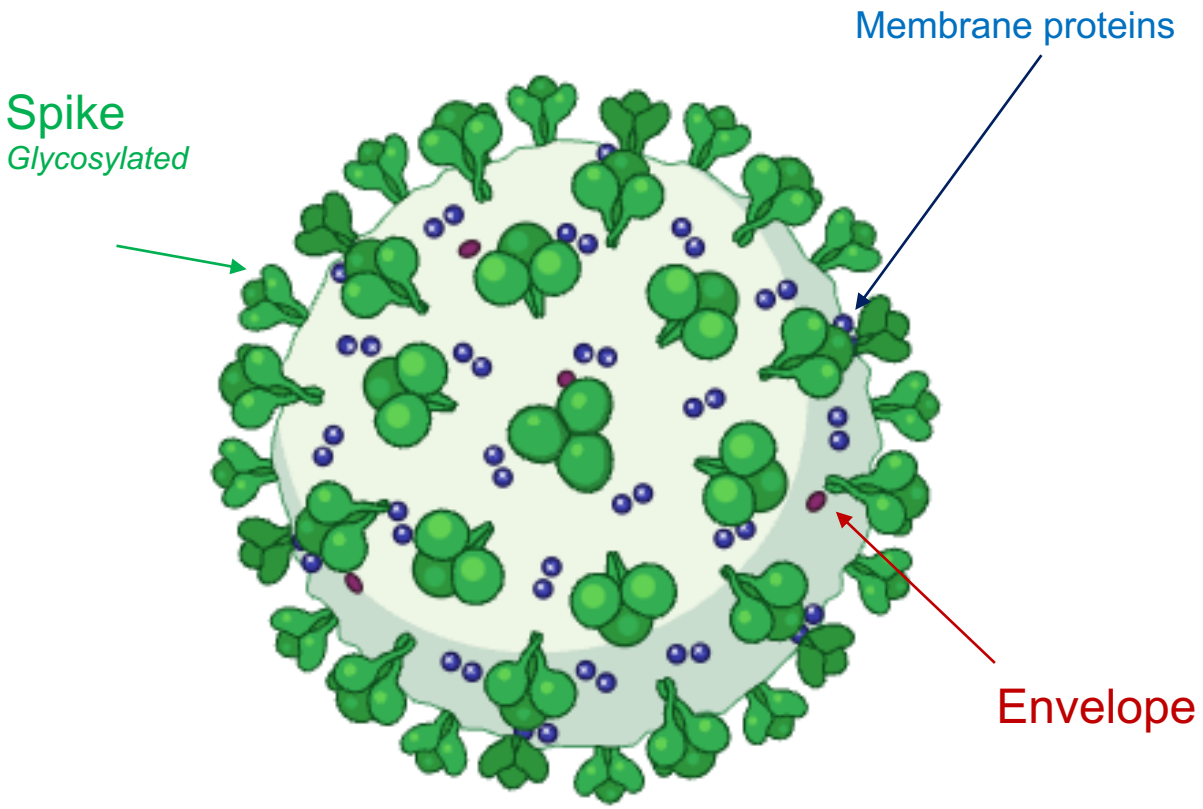
Genetics 564 2024

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

What is the hypothesis?

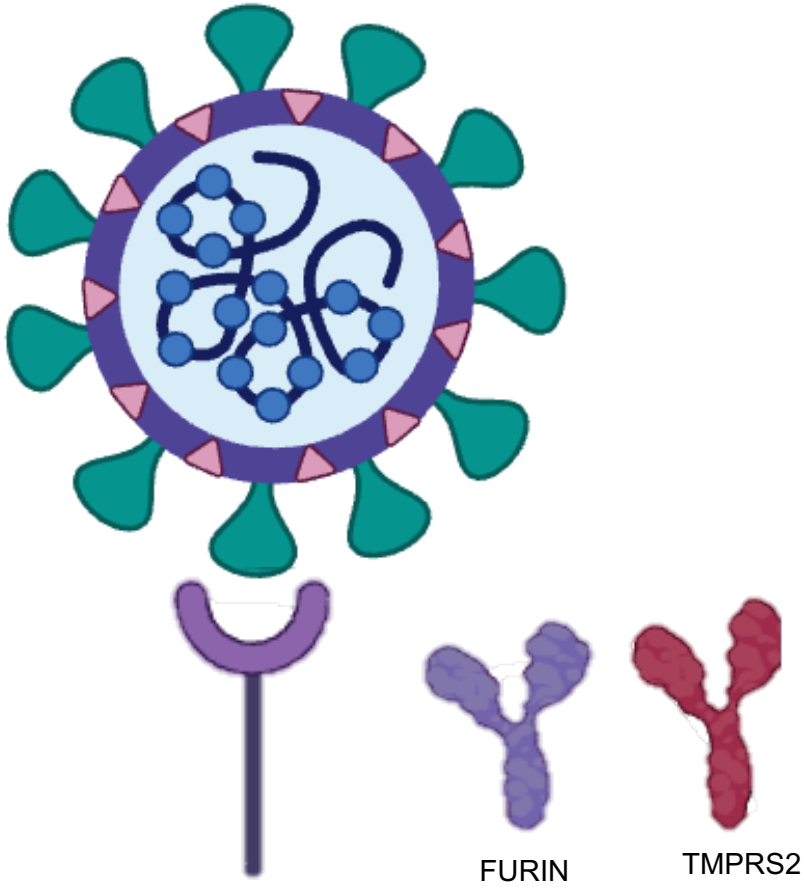
- Using genome-scale CRISPR loss of function test they should be able to find genes that grants/increase resistance to SARS-COV 2

What are the Life stages for SARS-COV- 2

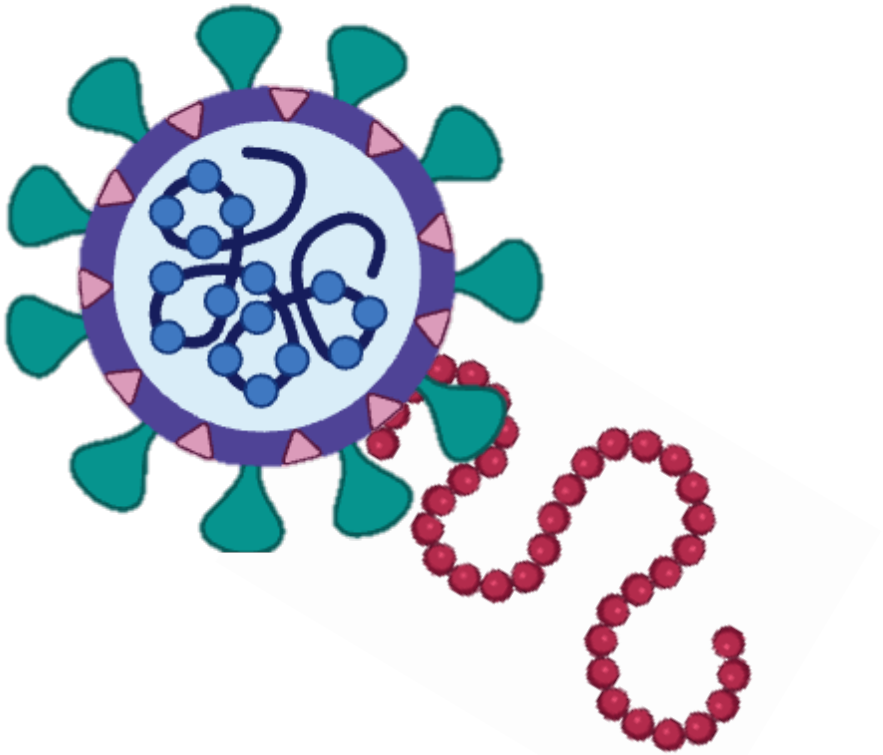


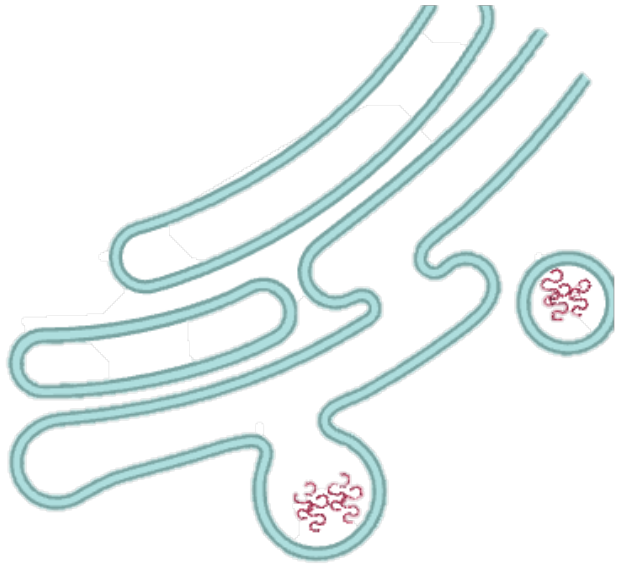
D416G

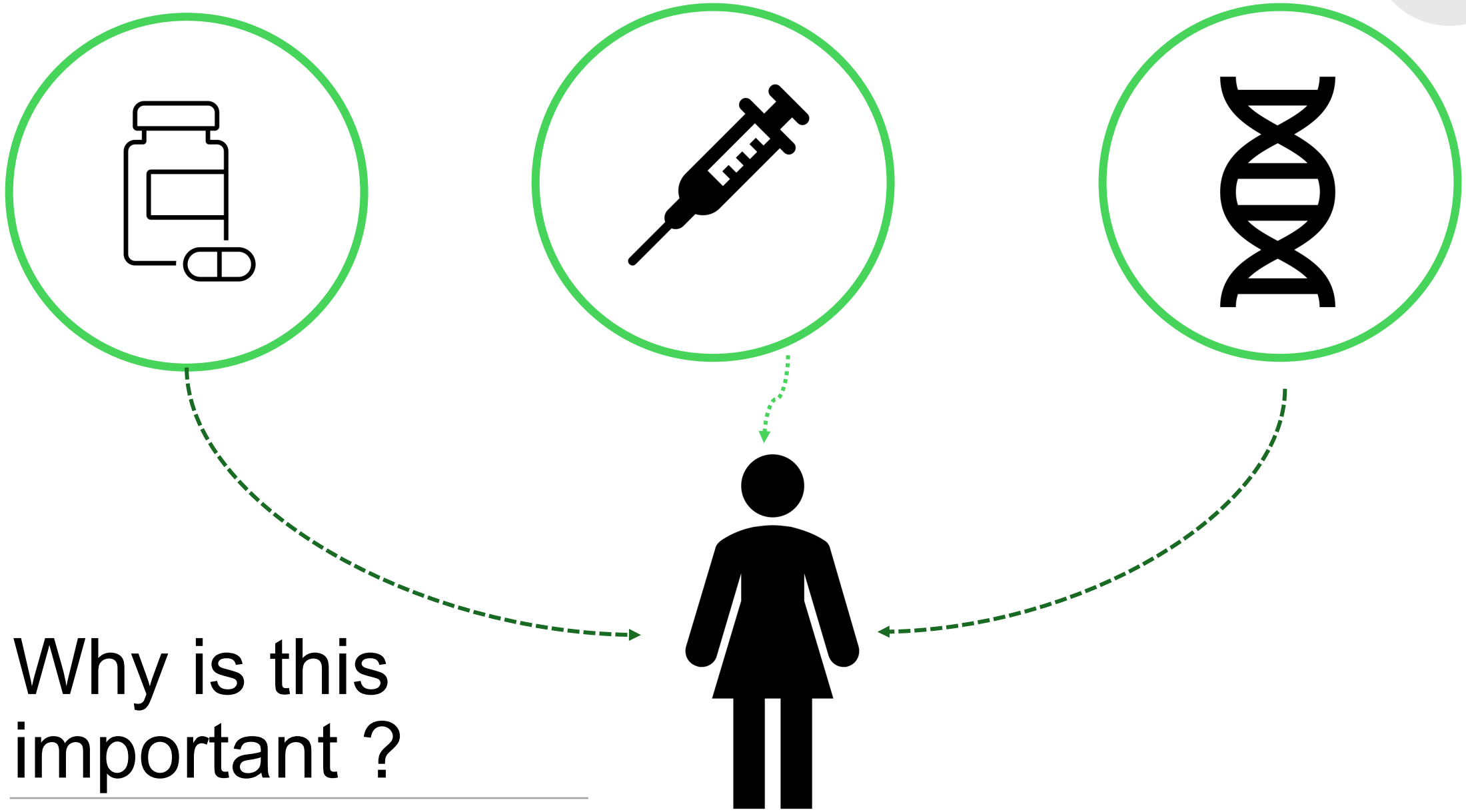
What are the life stages of COV-2? Continue



What are the life stages of COV-2? Continue



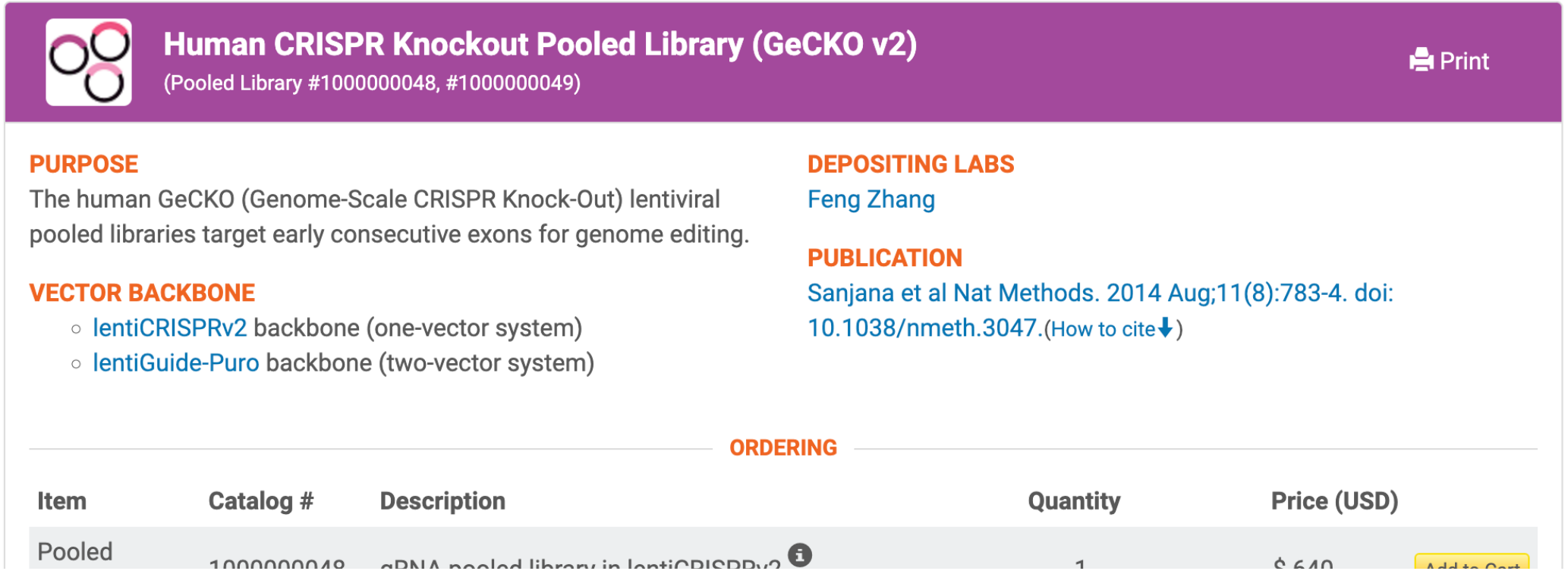




Why is this important ?

What are the methods?

GeCKOv2 CRISPR-Cas9 library



Human CRISPR Knockout Pooled Library (GeCKO v2)
(Pooled Library #1000000048, #1000000049)

Print

PURPOSE
The human GeCKO (Genome-Scale CRISPR Knock-Out) lentiviral pooled libraries target early consecutive exons for genome editing.

VECTOR BACKBONE
- lentiCRISPRv2 backbone (one-vector system)
- lentiGuide-Puro backbone (two-vector system)

DEPOSITING LABS
Feng Zhang

PUBLICATION
Sanjana et al Nat Methods. 2014 Aug;11(8):783-4. doi: 10.1038/nmeth.3047.(How to cite)

ORDERING

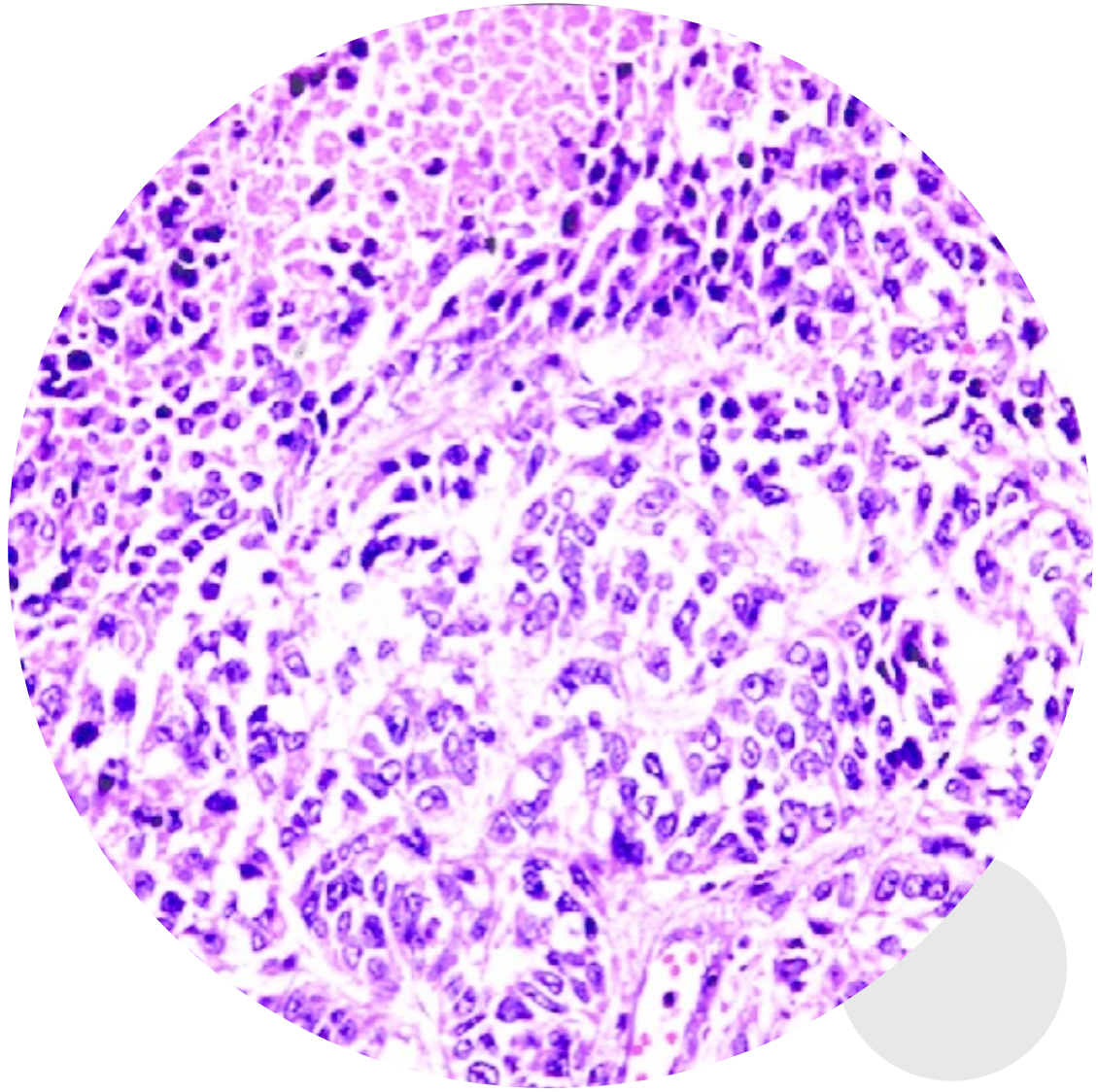
Item	Catalog #	Description	Quantity	Price (USD)
Pooled	1000000048	gRNA pooled library in lentiCRISPRv2	1	$ 640

Human alveolar cell carcinoma

A540^ACE2

Located in the alveoli

reduces surface tension and prevents alveolar collapse during ventilation.

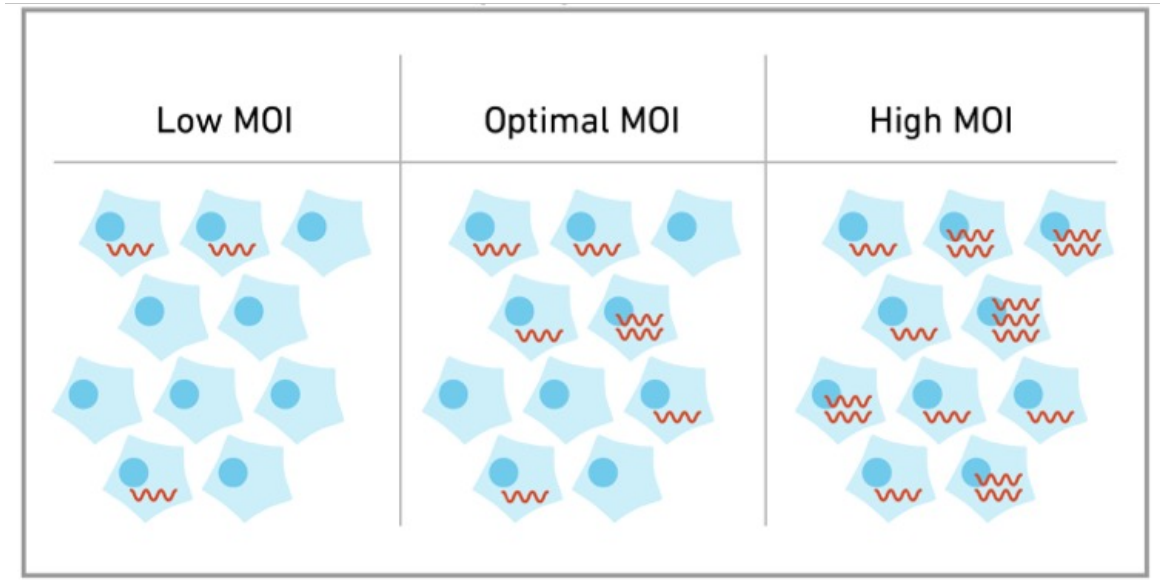


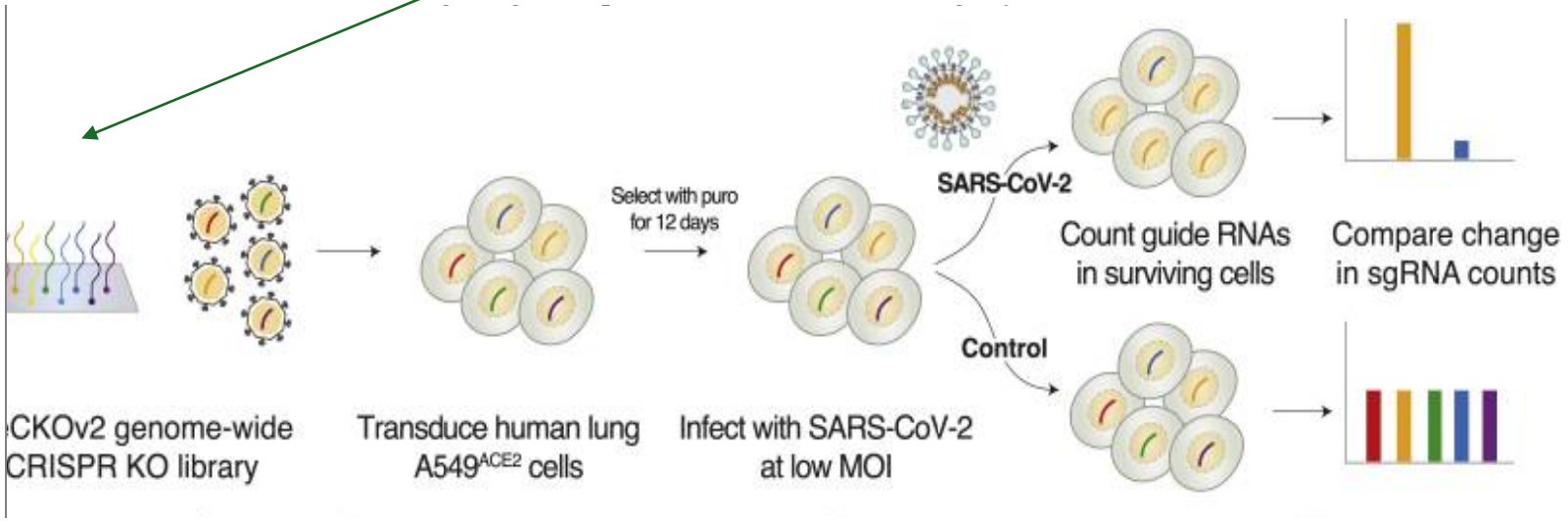
How do we start?

$$P(k)=e^{-\lambda}\lambda/k!$$

λ=MOI

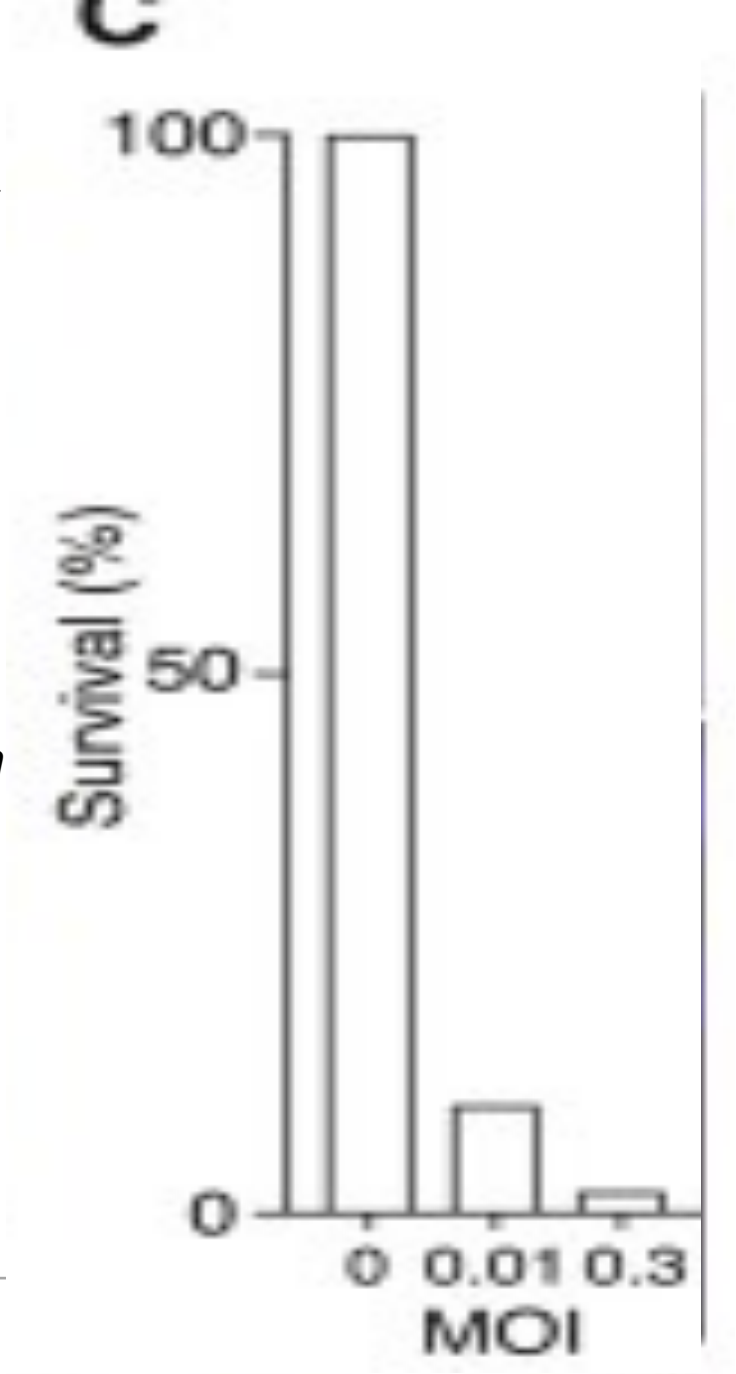
K=# of viral particles and take values

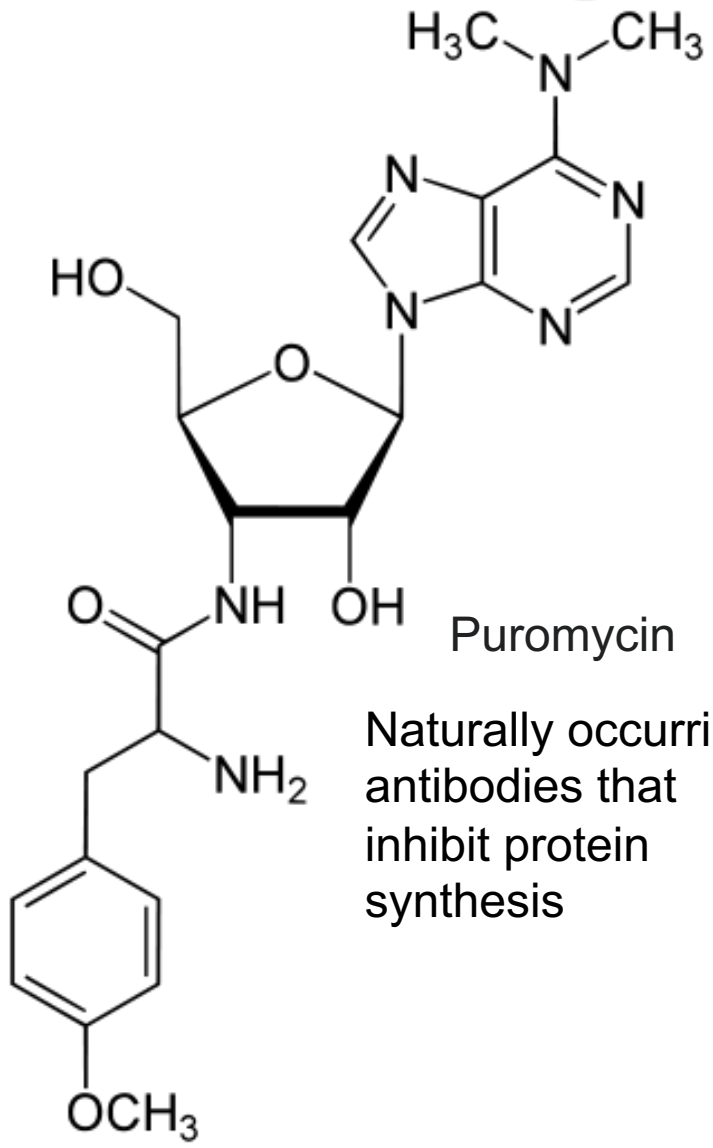




What is tra continue

1. Selection
2. Breading
3. Infection (*Moi = .3 between*
4. stained

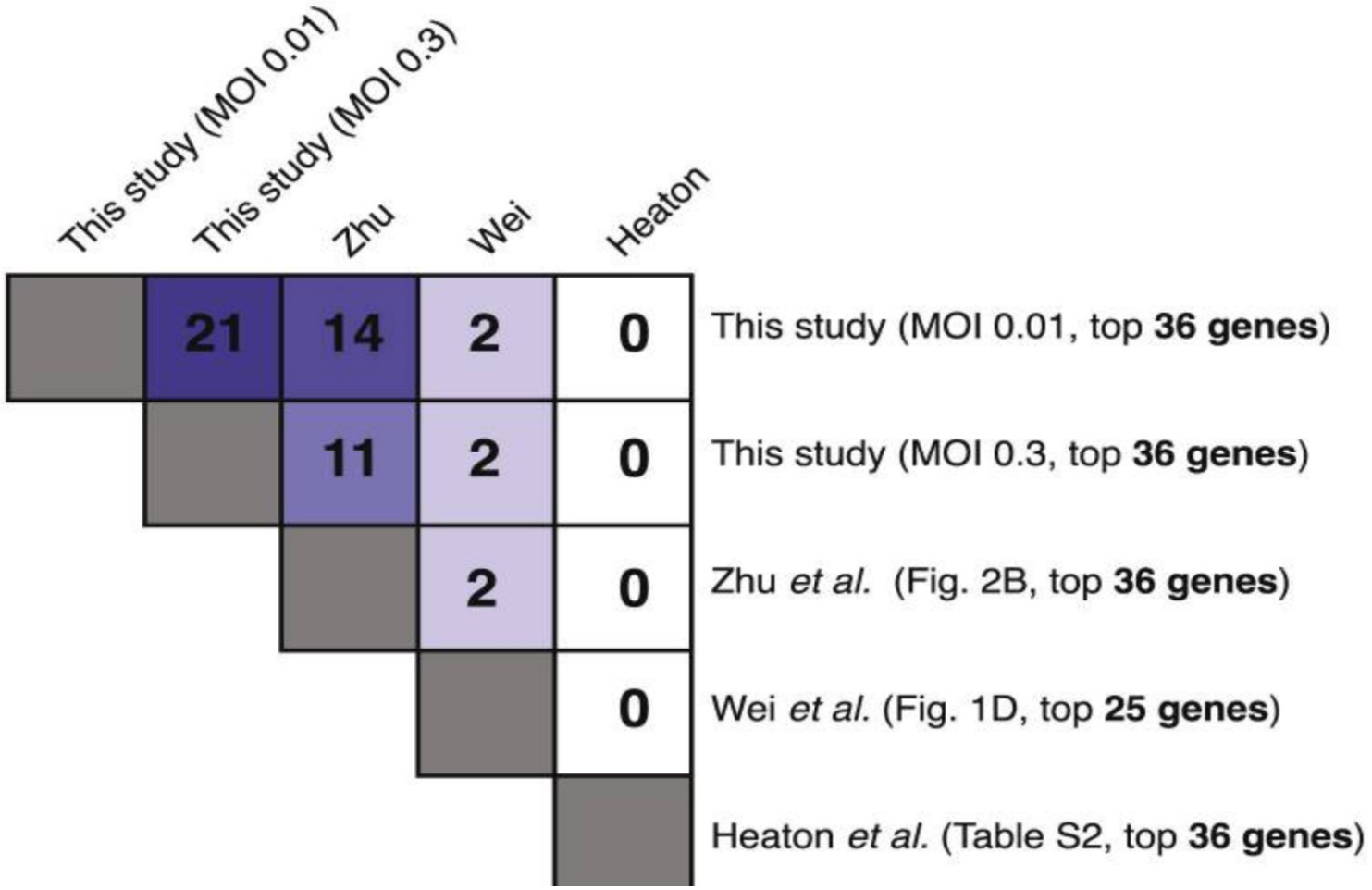




Puromycin

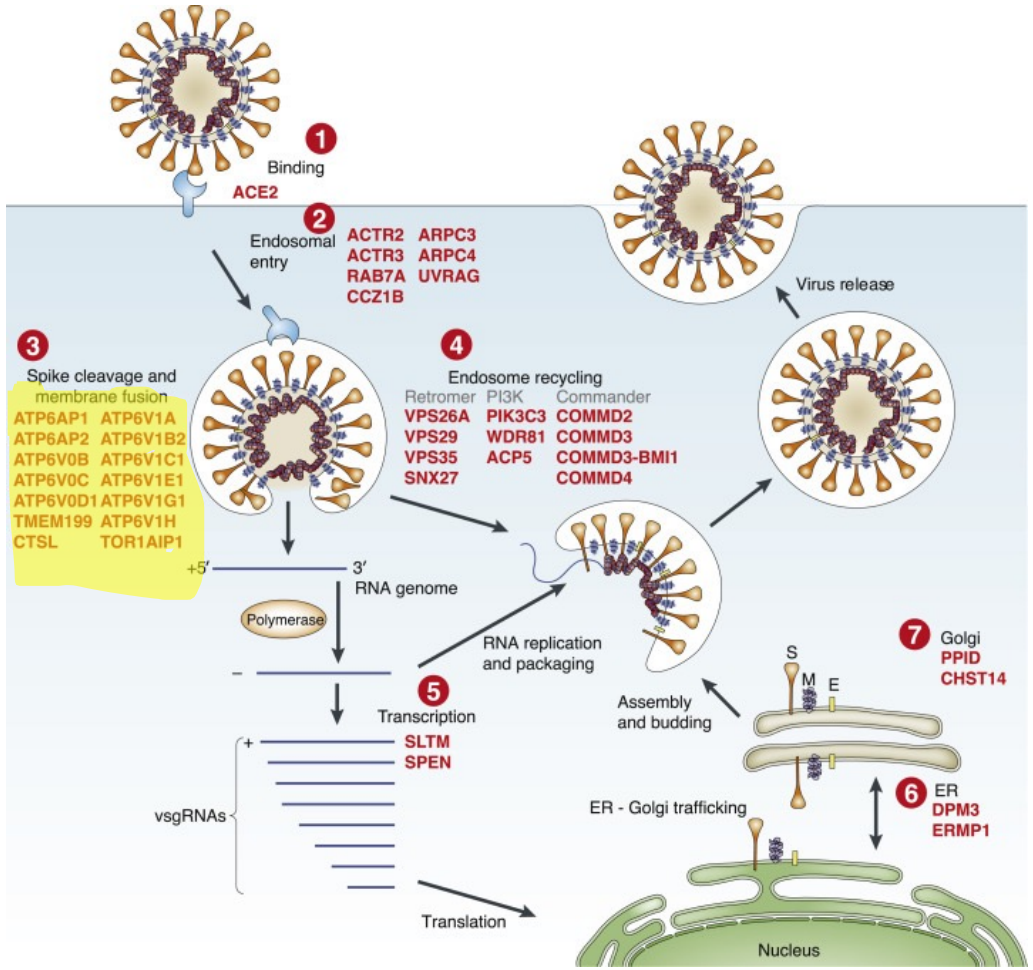
Naturally occurring antibodies that inhibit protein synthesis

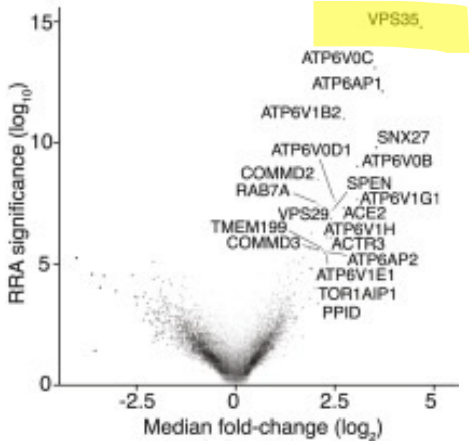
How does this compare to others' research?



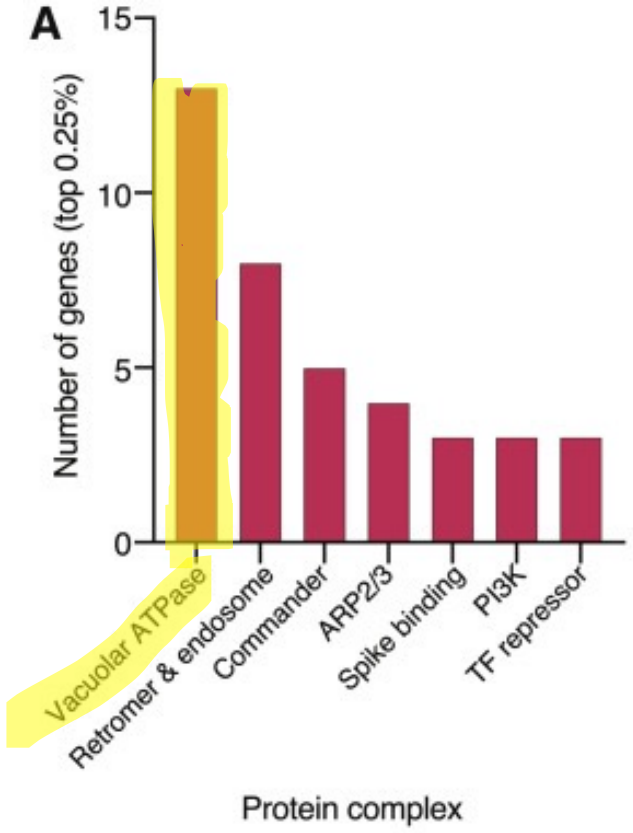
We have the genes now what?

Where are the target genes?

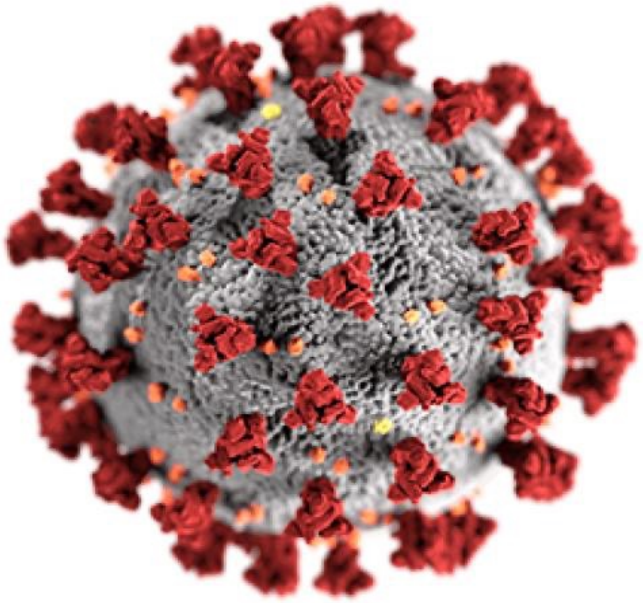




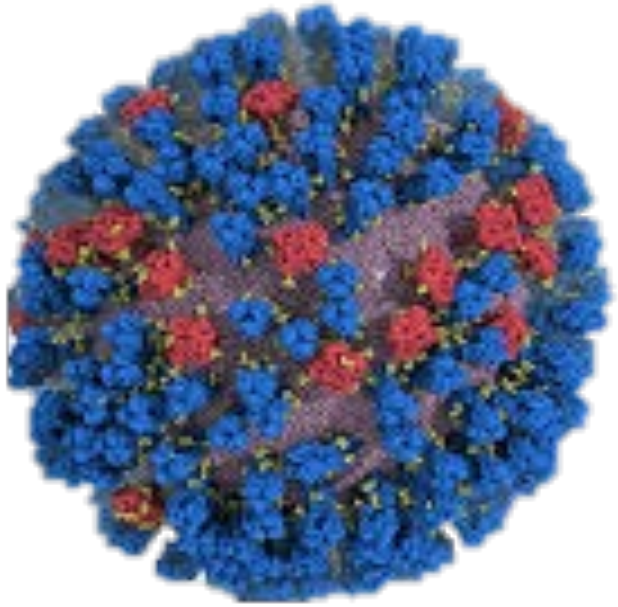
Quick comment



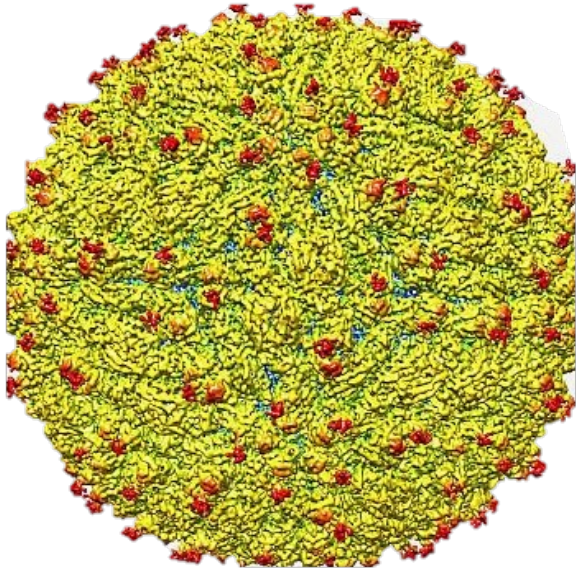
Can we use this information for other viruses?



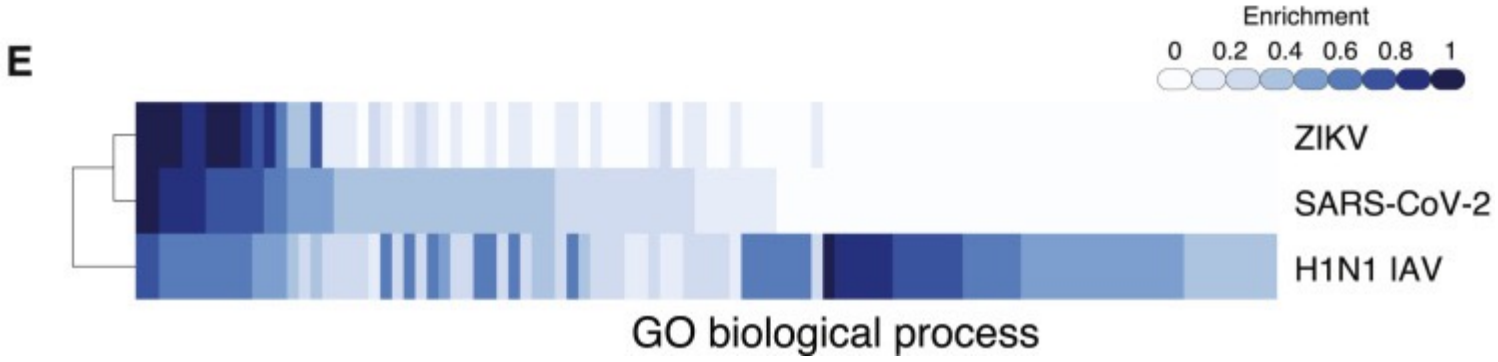
SARS-COV-2



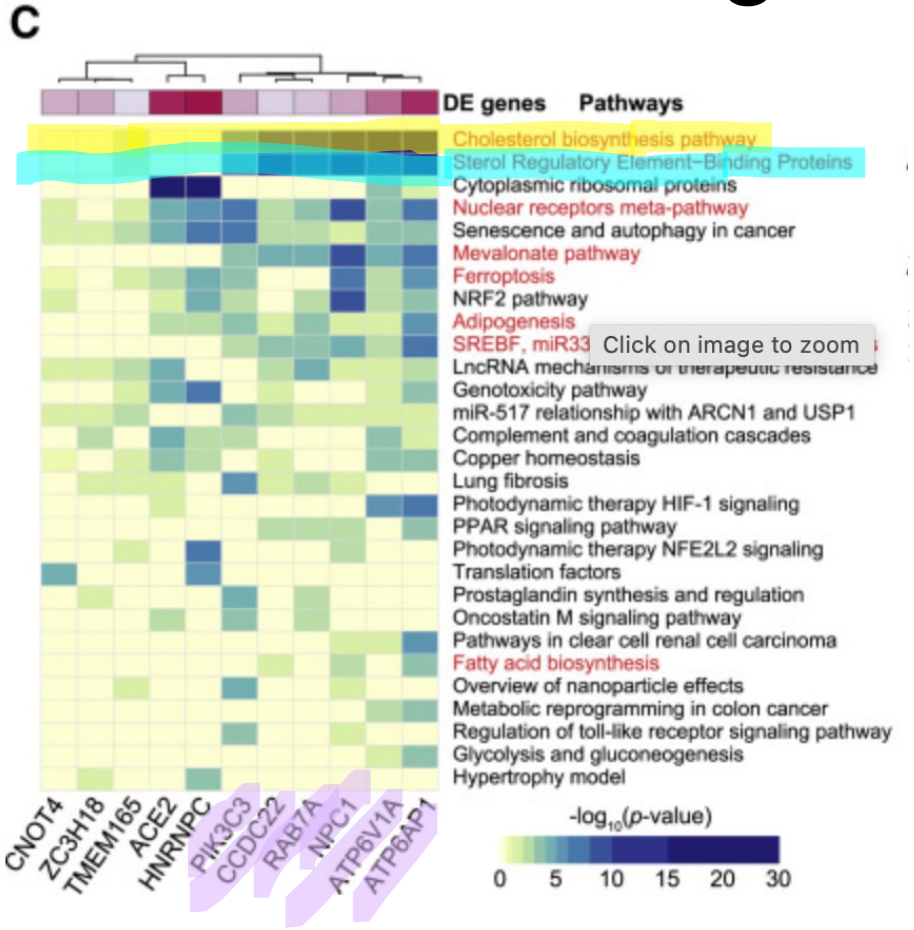
AIV



ZIKA



Was there a significant pathway?



Front Physiol. 2021; 12: 750544.
Published online 2021 Nov 11. doi: 10.3389/fphys.2021.750544
PMCID: PMC8632007
PMID: 34858206

Endosomal Cholesterol in Viral Infections – A Common Denominator?

Mirco Glitscher and Eberhard Hildt *

Abstract

Go to:

J Biol Chem. 2023 Jun; 299(6): 104763.
Published online 2023 Apr 28. doi: 10.1016/j.jbc.2023.104763
PMCID: PMC10140059
PMID: 37119851

The role of high cholesterol in SARS-CoV-2 infectivity

Hao Wang,[1,2,3] Zixuan Yuan,[1,2,3] Mahmud Arif Pavel,[1,2] Sonia Mediouni Jablonski,[4] Joseph Jablonski,[4] Robert Hobson,[5,6] Susana Valente,[4] Chakravarthy B. Reddy,[7] and Scott B. Hansen[1,2,*]

Summary

- 30 host genes found
- 6 of the genes shared a mechanism as the cholesterol pathway
- Upregulating cholesterol levels block SARS
- An increase in cholesterol leads to higher resistance
- Rab7a regulates cell surface expression of ACE

Future advancements and understandings

- Understanding cholesterol biosynthesis pathway and SARS relationship
- Possibility of other lipids affecting SARS
- Understanding Rab7a blocking of SARS
- PiKC3 drug target


End of Presentation
ANY QUESTIONS?
memecreator.org

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