

¿Cómo enseñar edición genética en las aulas?

@LluísMontoliu

CNB-CSIC y CIBERER-ISCIII, Madrid



UA

Francisco Juan Martínez Mojica

1993



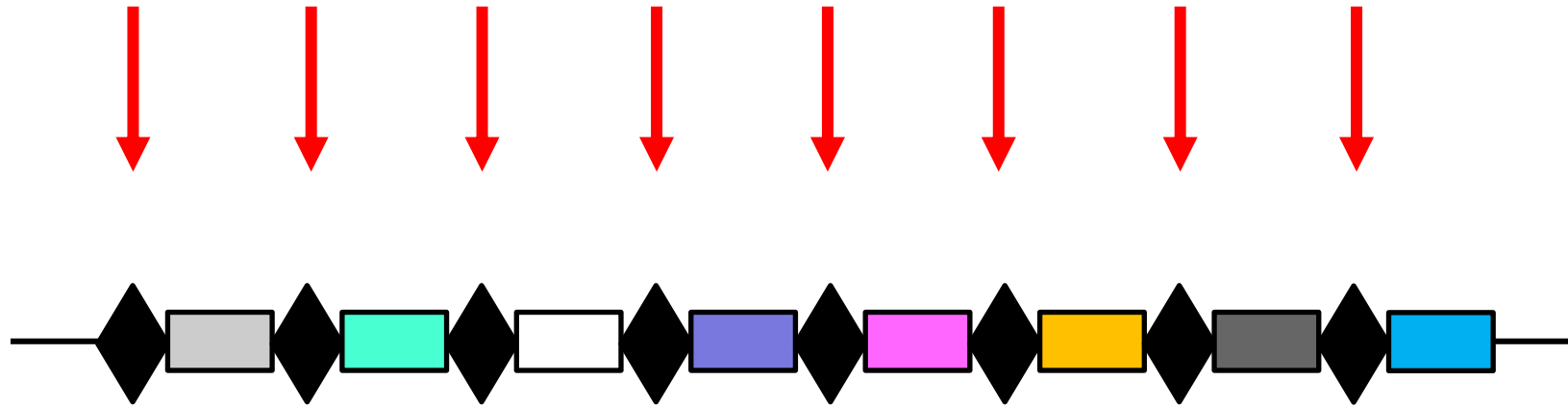
elpais.com

Salinas de Santa Pola (Alicante)

ADN



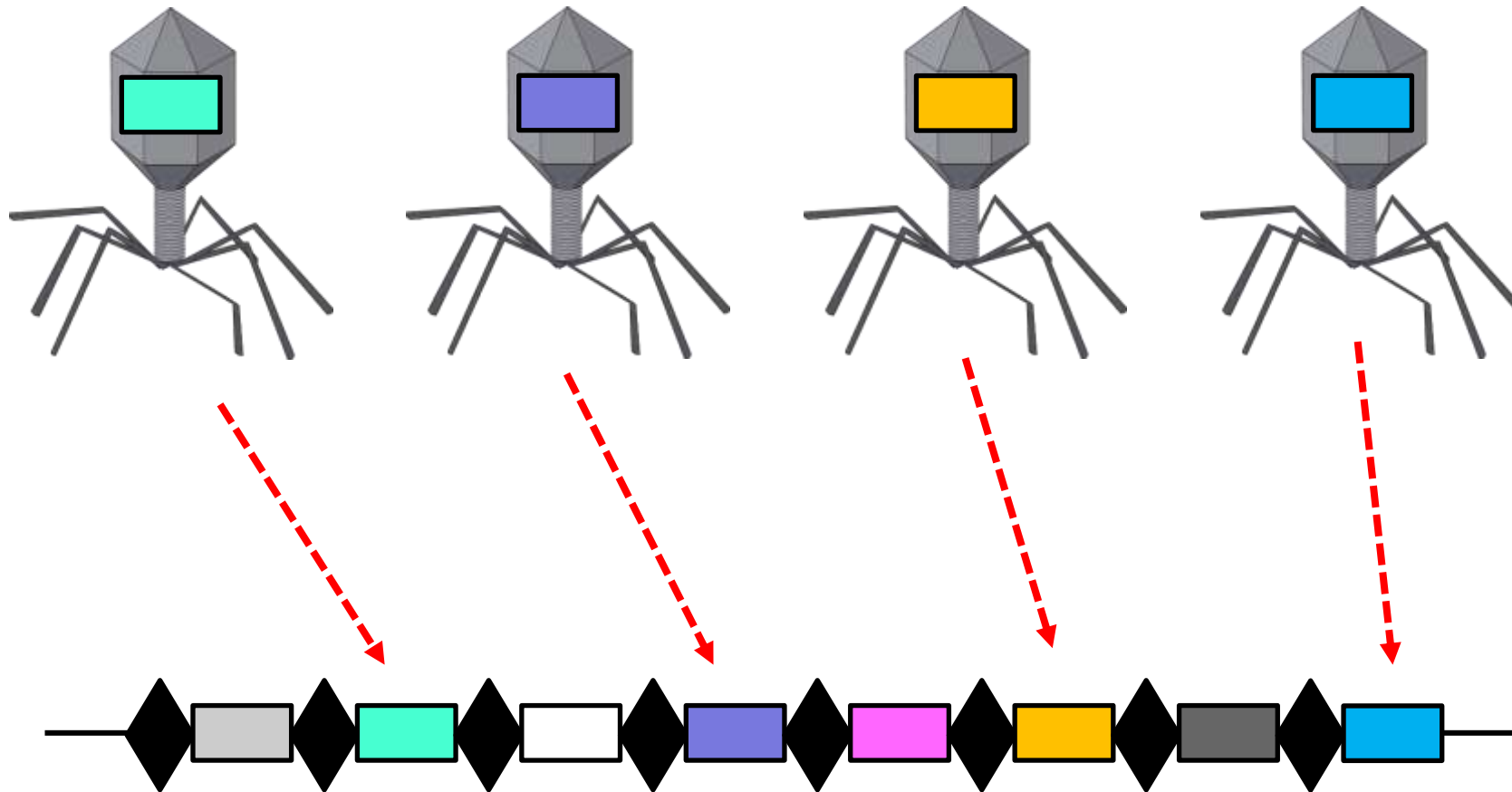
2001 - 2002



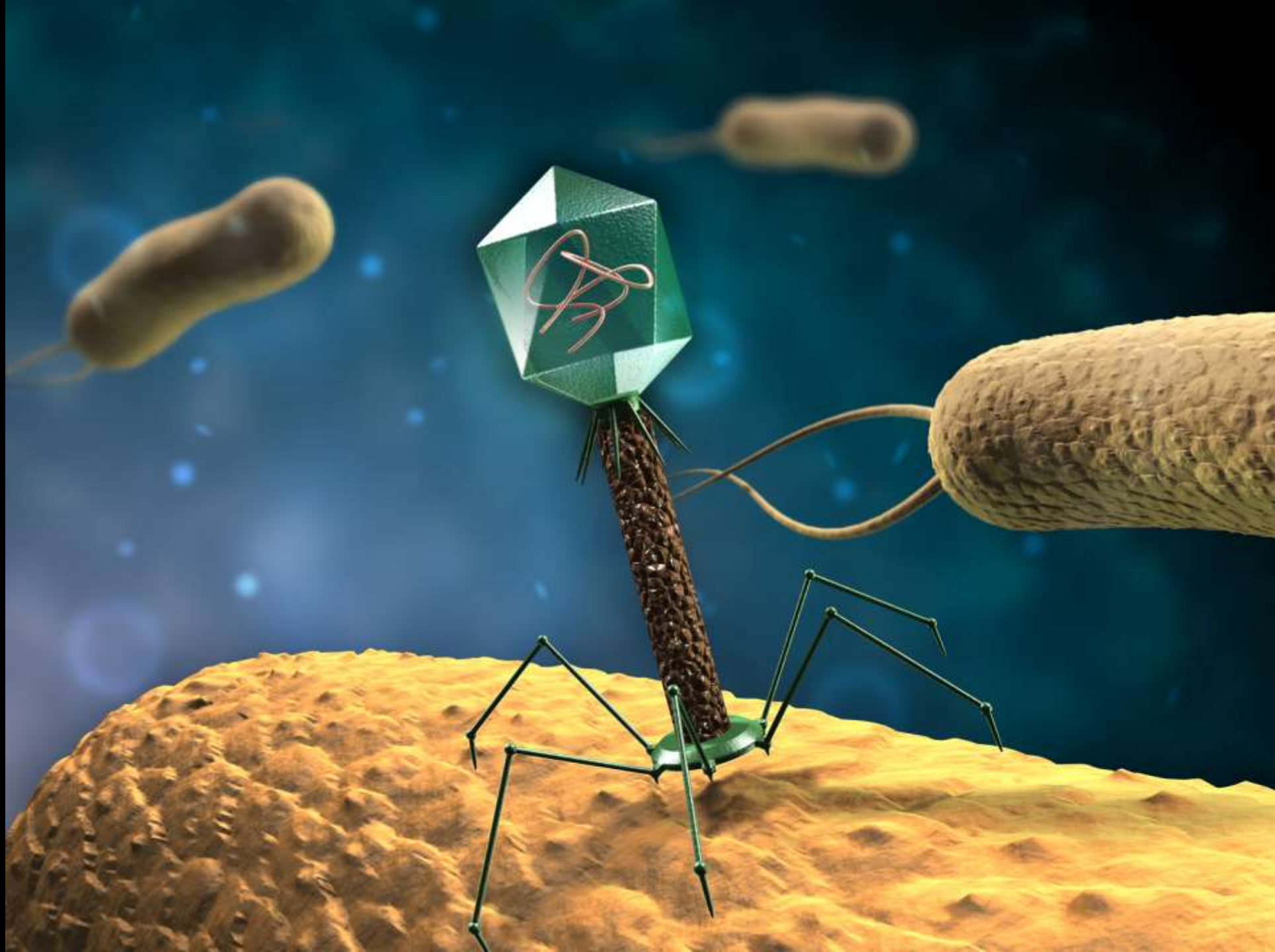
C.R.I.S.P.R. (*crísper*)

Clustered Regularly Interspaced Short Palindromic Repeats

Repeticiones cortas palindrómicas agrupadas y regularmente espaciadas



2003 - 2005



Emmanuelle Charpentier

Jennifer Doudna



1993



2013



Francisco Mojica
University of Alicante, Spain



Rodolphe Barrangou
North Carolina State Univ, Raleigh, USA



Philippe Horvath
DuPont Nutrition and Health, France



Luciano Marraffini
The Rockefeller Univ, New York, USA



John van der Oost
Wageningen University, The Netherlands



Emmanuelle Charpentier
MPI for Infect. Biol., Berlin, Germany



Jennifer Doudna
Univ California Berkeley, CA, USA



Virginijus Siksnys
Vilnius University, Lithuania



Feng Zhang
BROAD-MIT, Cambridge, MA, USA



George Church
Harvard Med School, Boston, MA, USA



Rudolf Jaenisch
Whitehead Inst, Cambridge, MA, USA

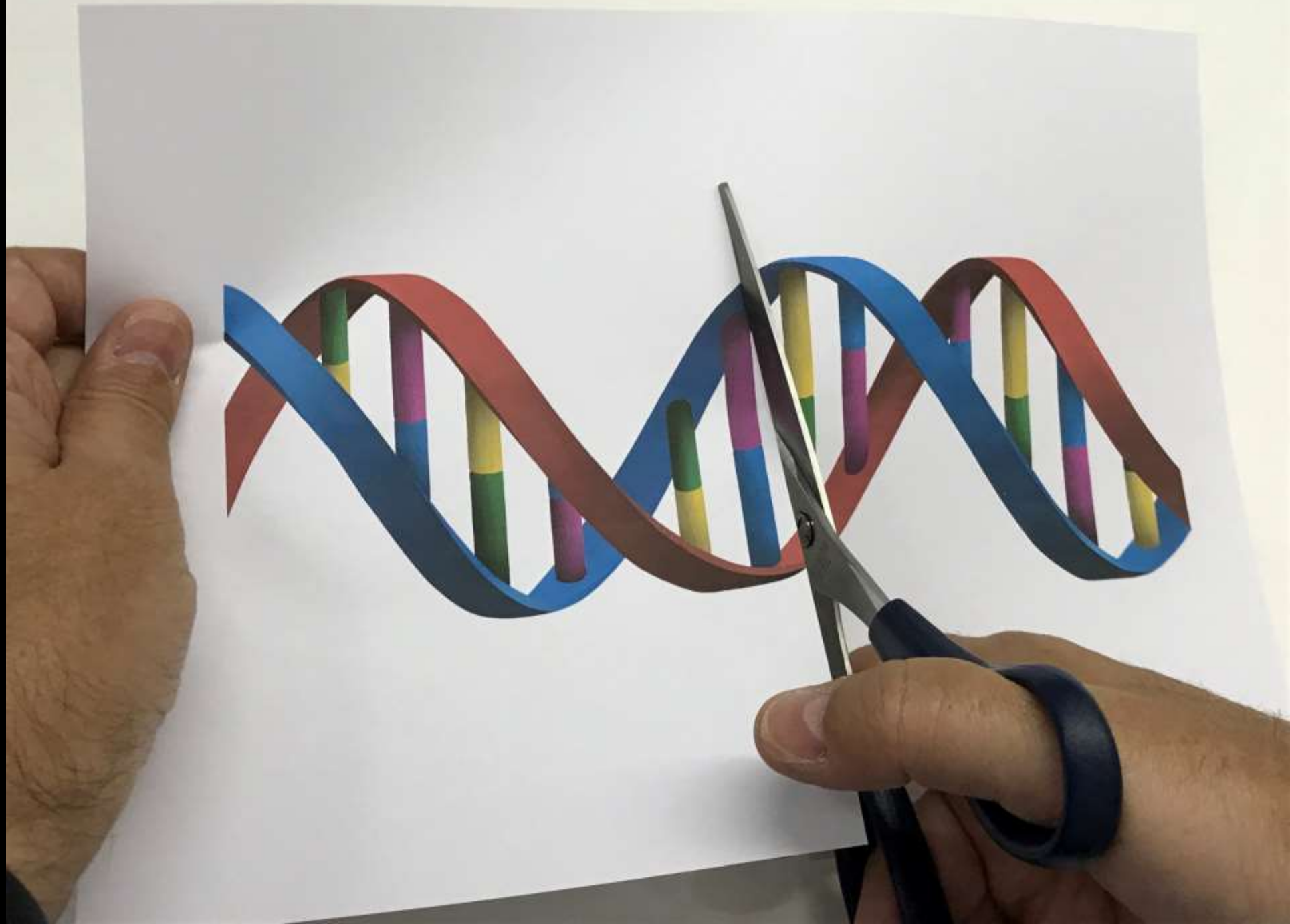


J. Keith Joung
Mass Gen Hosp, Charlestown, MA, USA

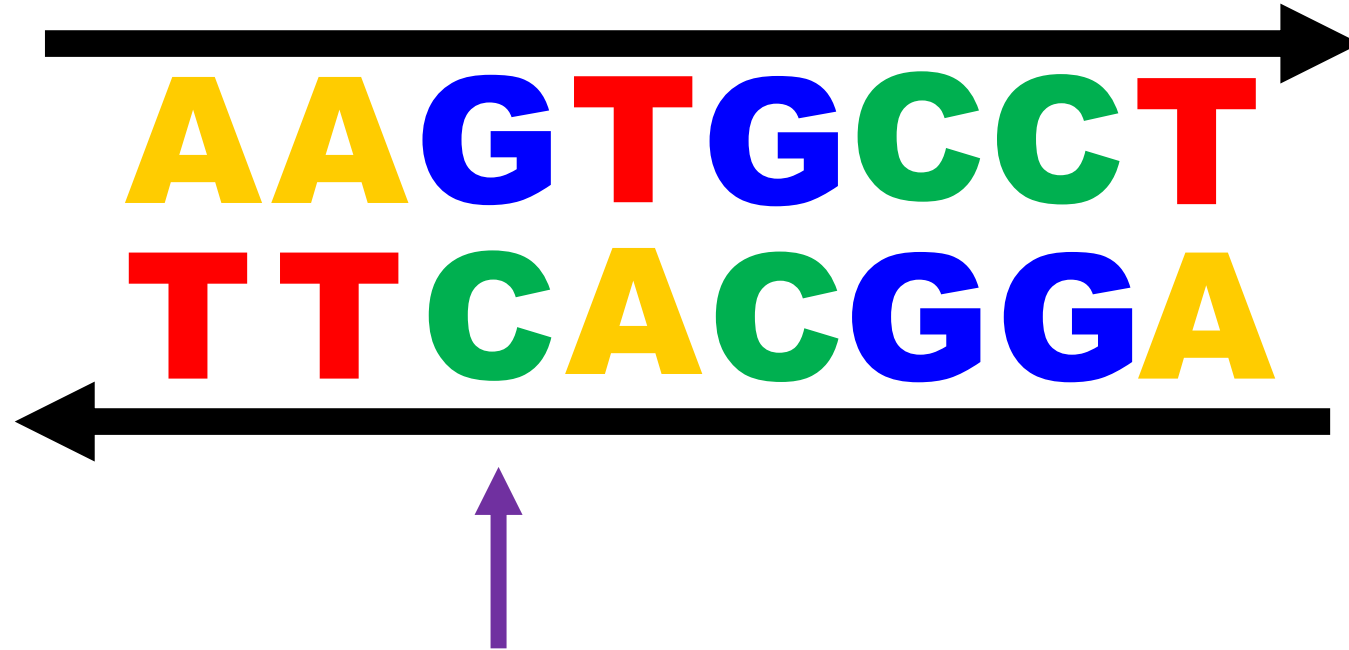


David R. Liu
BROAD-MIT, USA

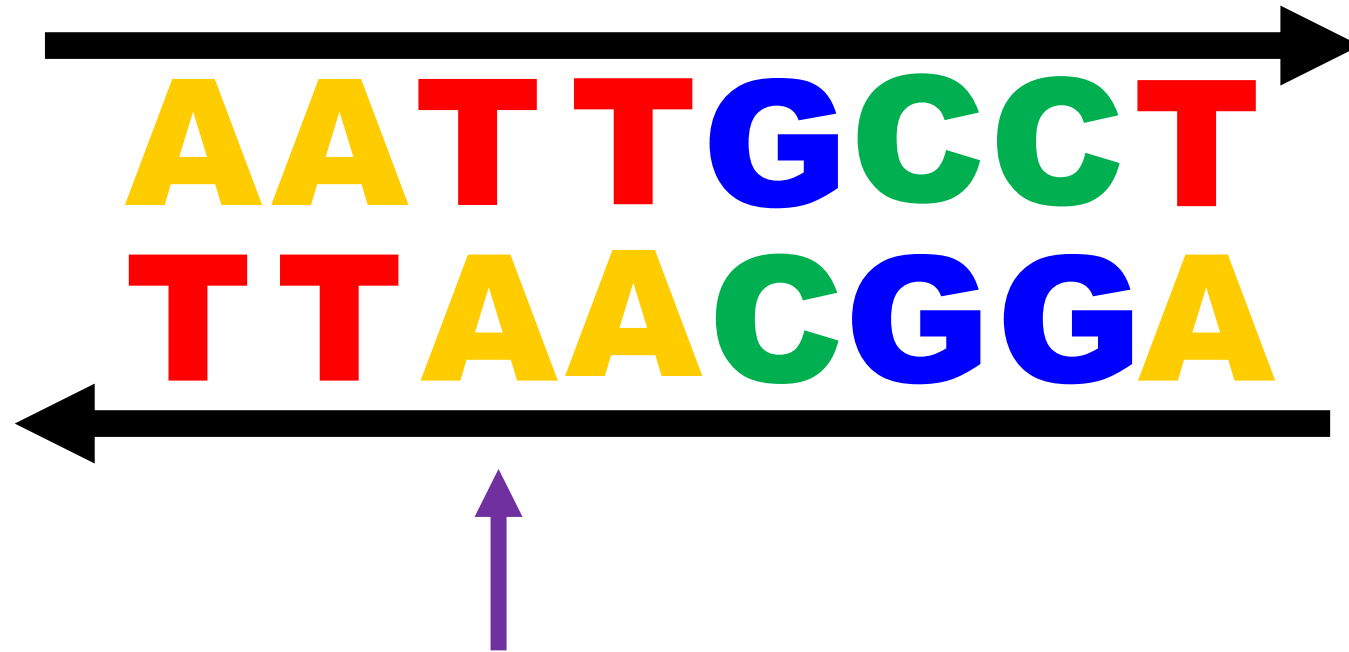
2016

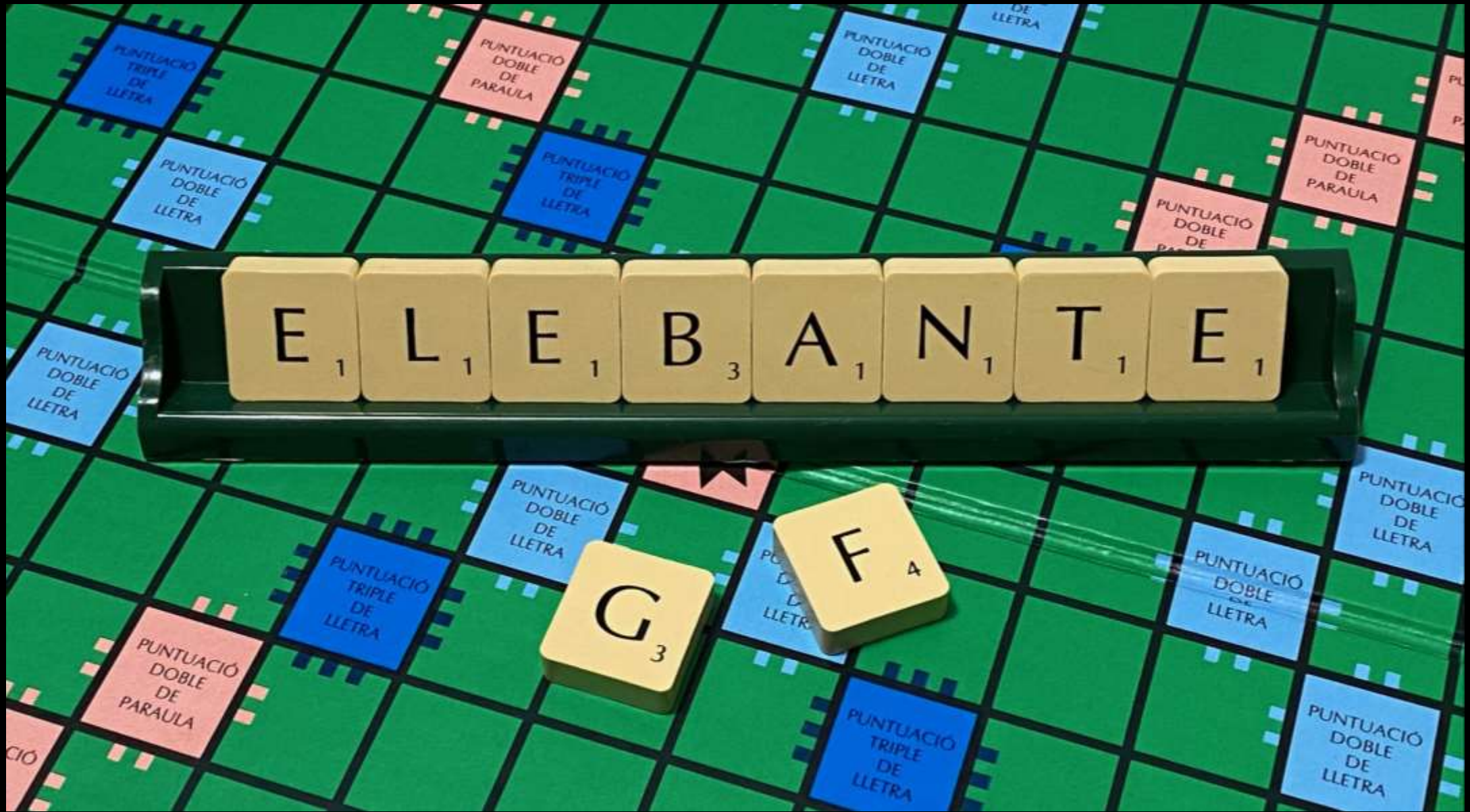


ADN



ADN



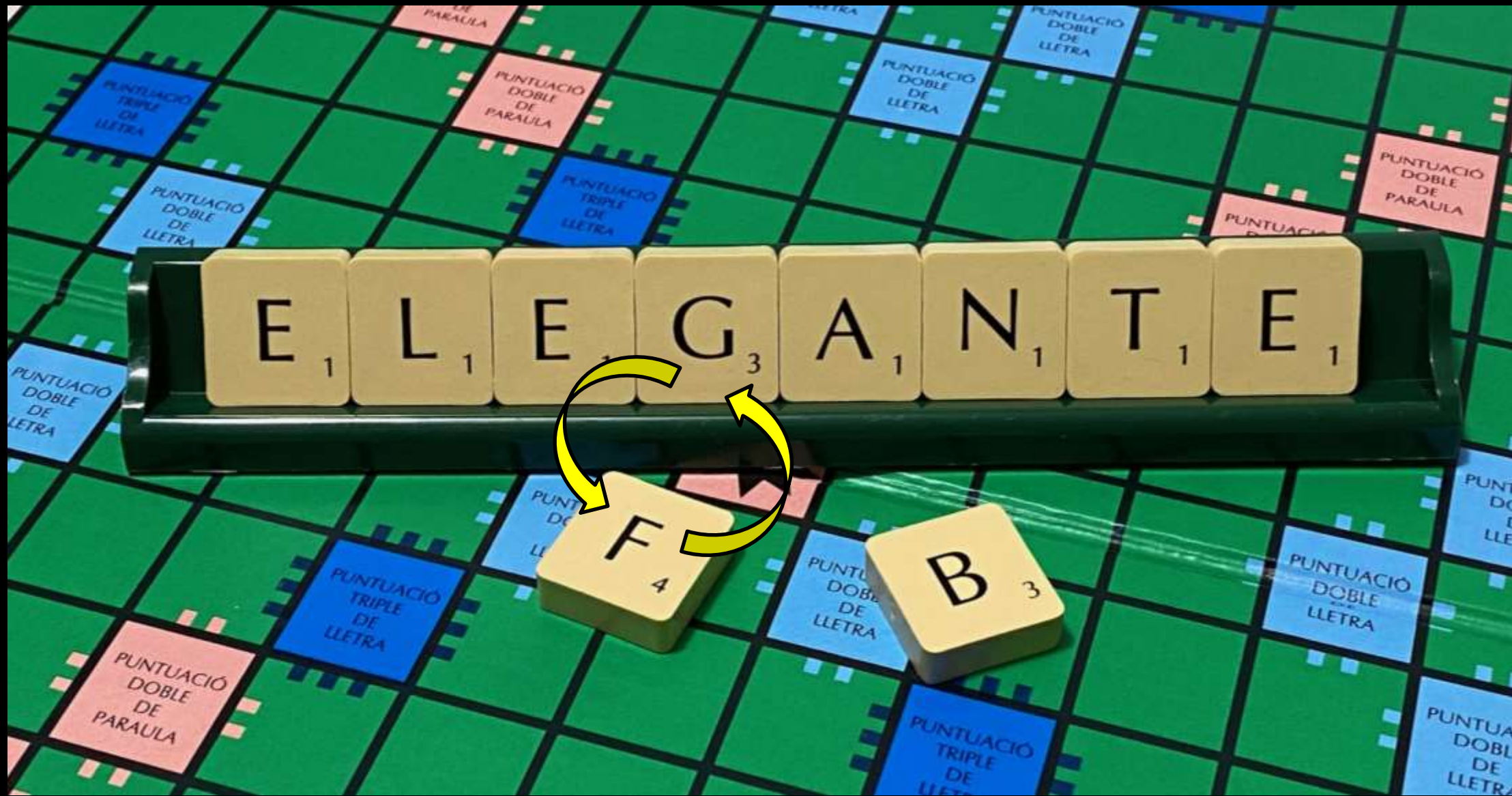


E₁ L₁ E₁ B₃ A₁ N₁ T₁ E₁

G₃

F₄





Burgos es una de las cumas de la humanidad

Burgos es una de las cumas de la humanidad



n



Burgos es una de las cu-as de la humanidad

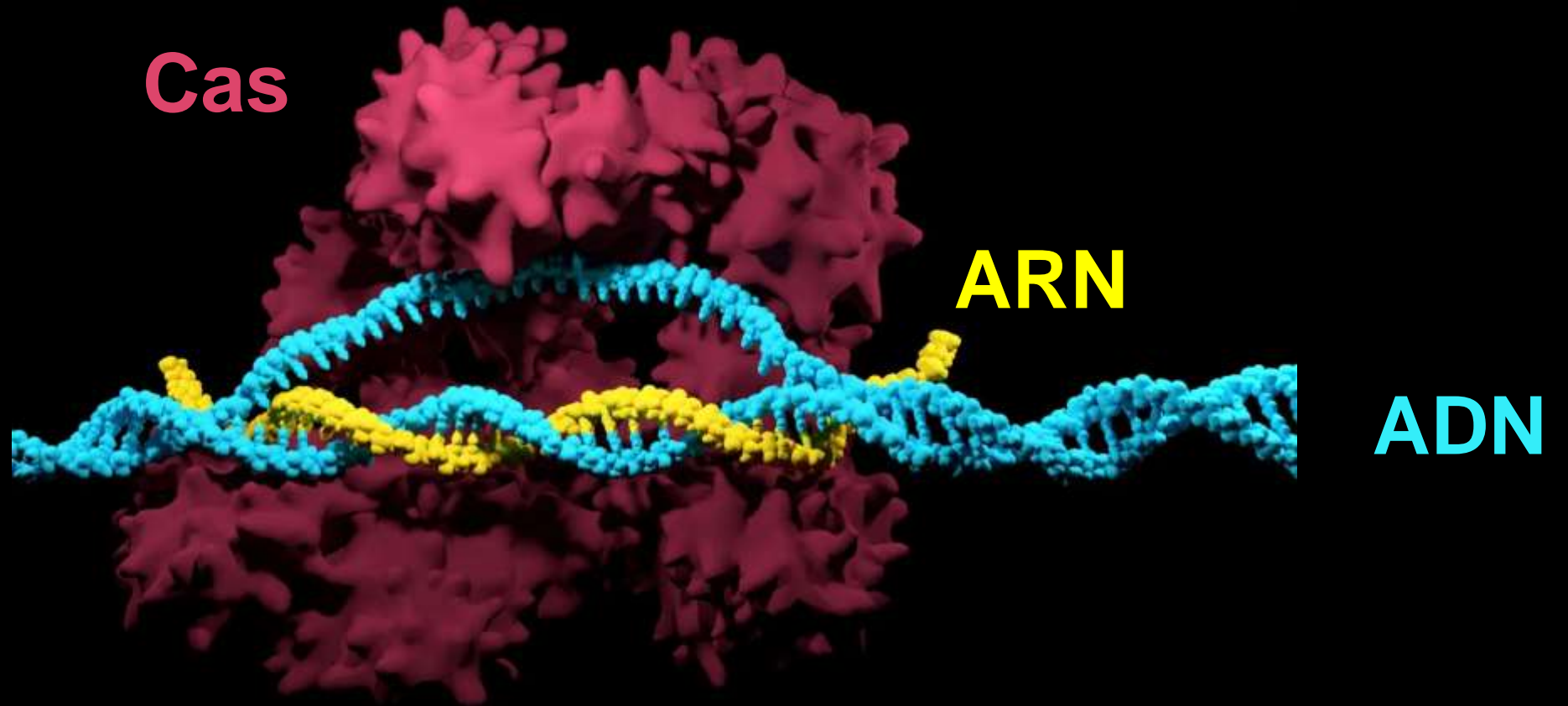


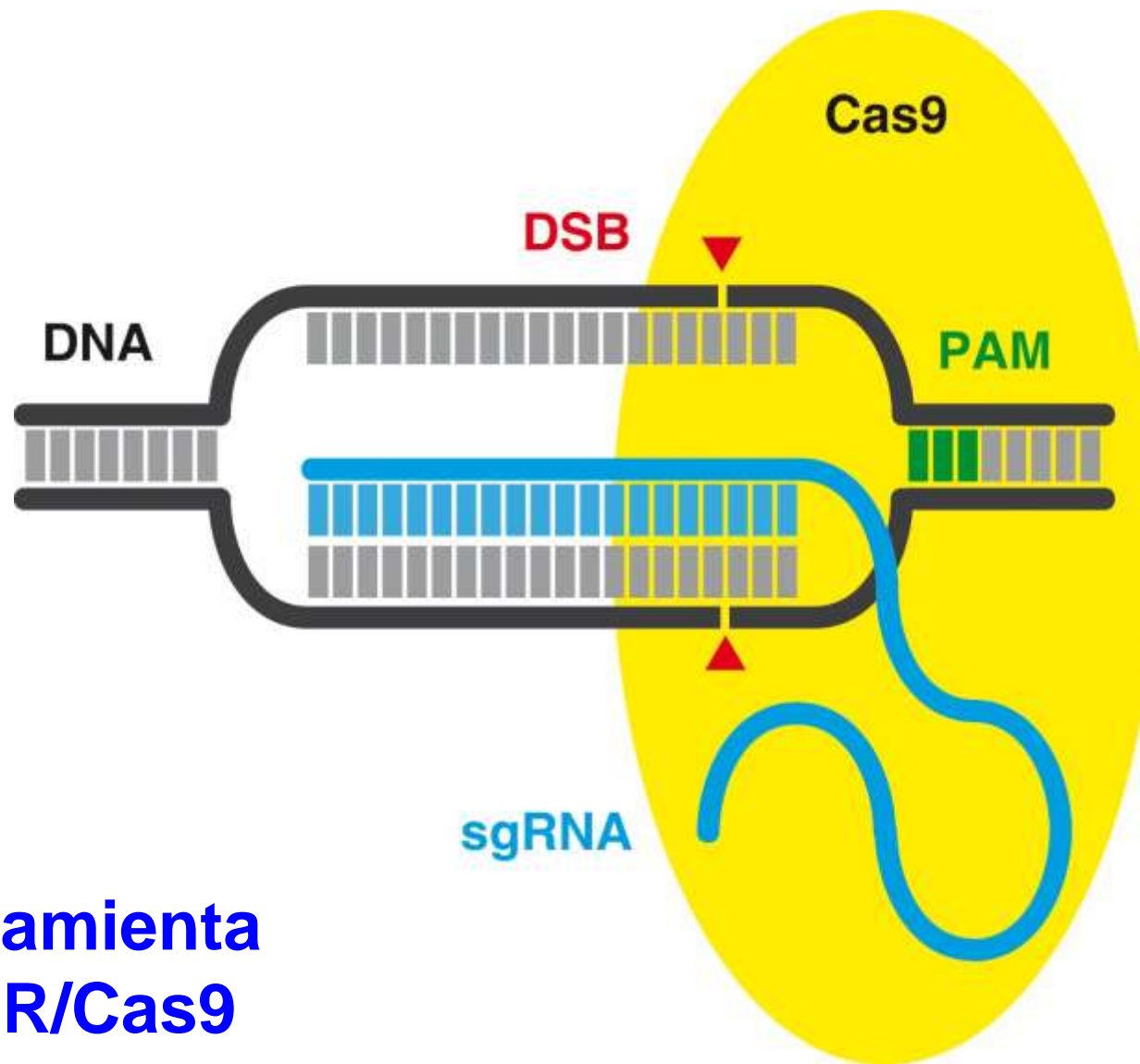
Burgos es una de las cunas de la humanidad

Burgos es una de las cunas de la humanidad



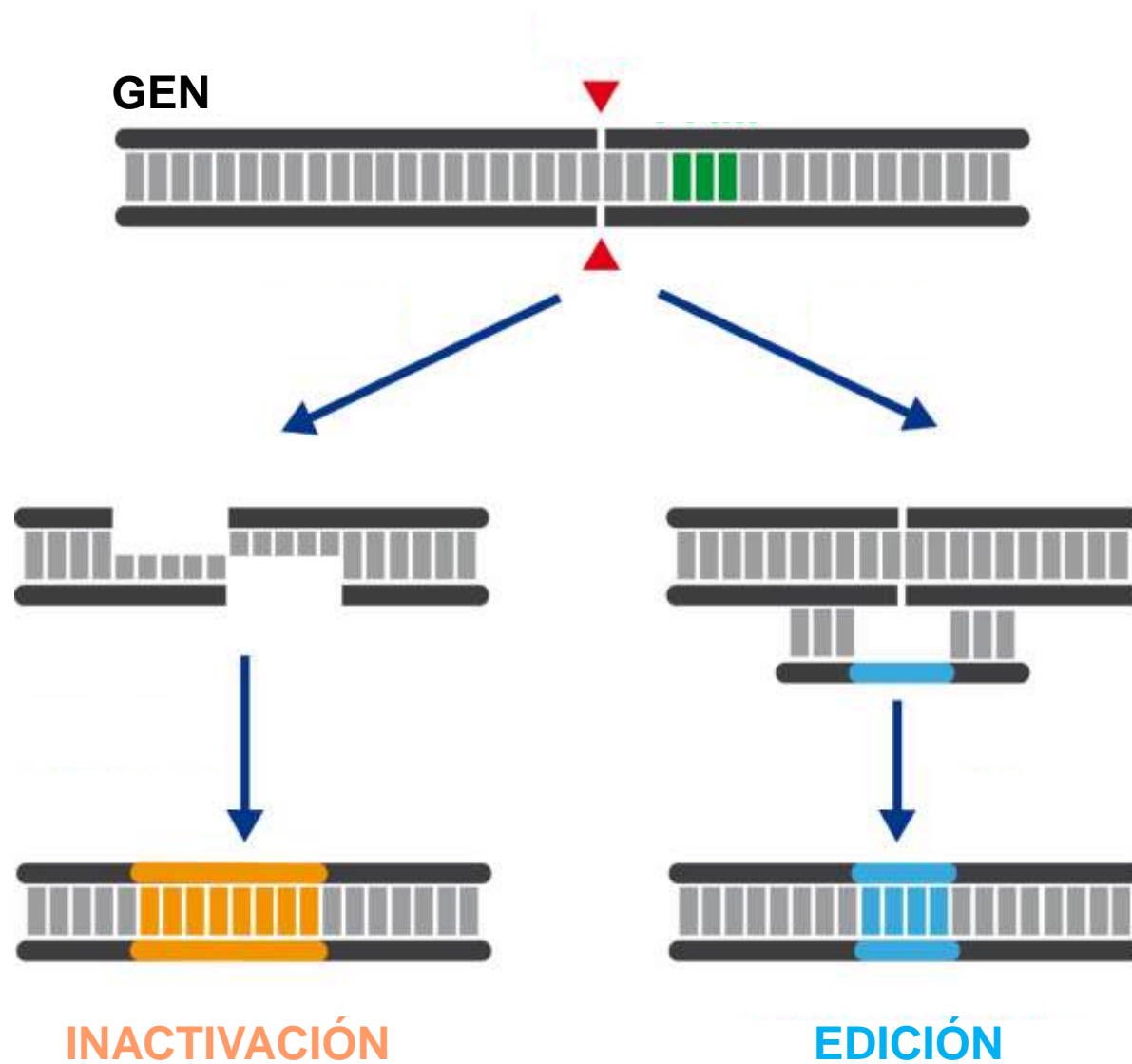
¿Cómo funcionan las CRISPR?





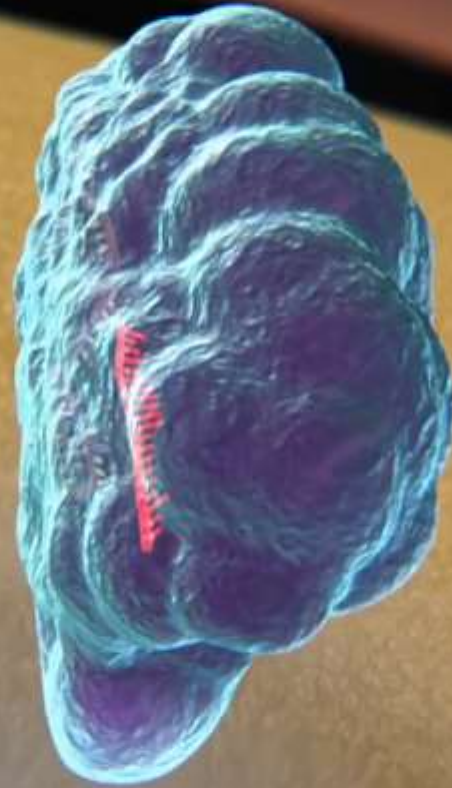
La herramienta
CRISPR/Cas9
original

¿Cómo funcionan las CRISPR?

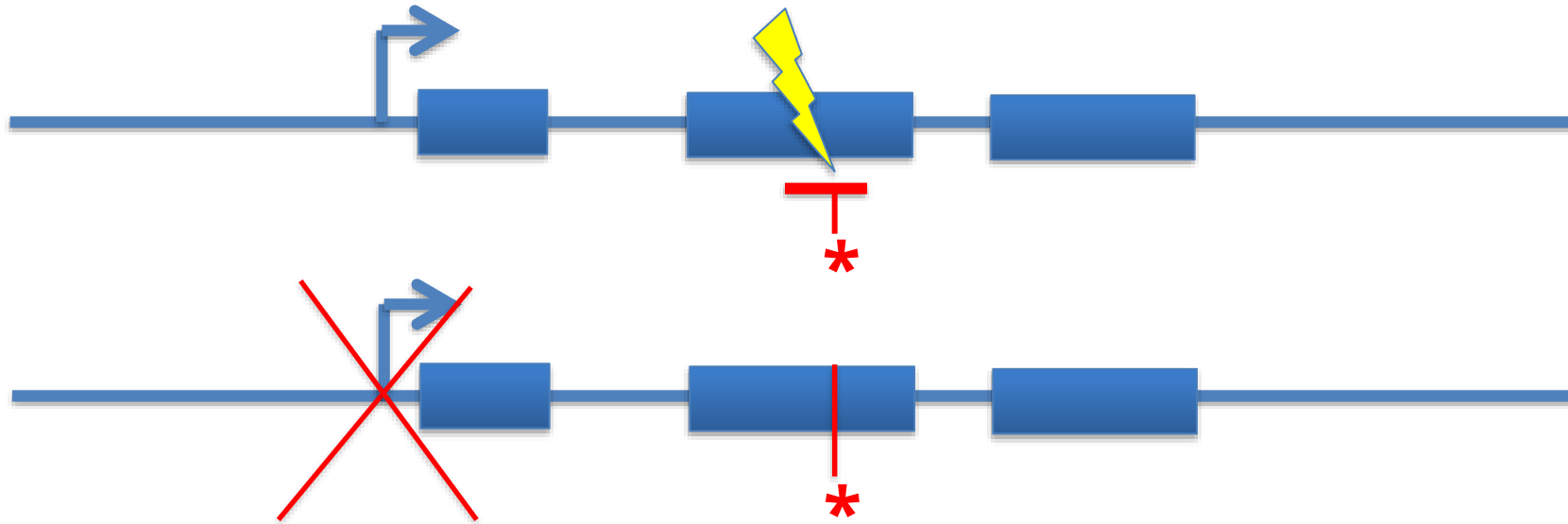


¿Cómo funciona la edición genética?

Movie by McGovern Institute for Brain Research at MIT



Mutaciones puntuales



Ratones CRISPR “avatar”



Modelos animales de albinismo





OCA1 (TYR) – OCA1A





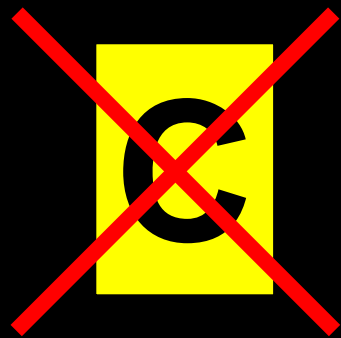
OCA1 (*TYR*) – OCA1B





OCA2 (OCA2)





OCA4 (*SLC45A2*)



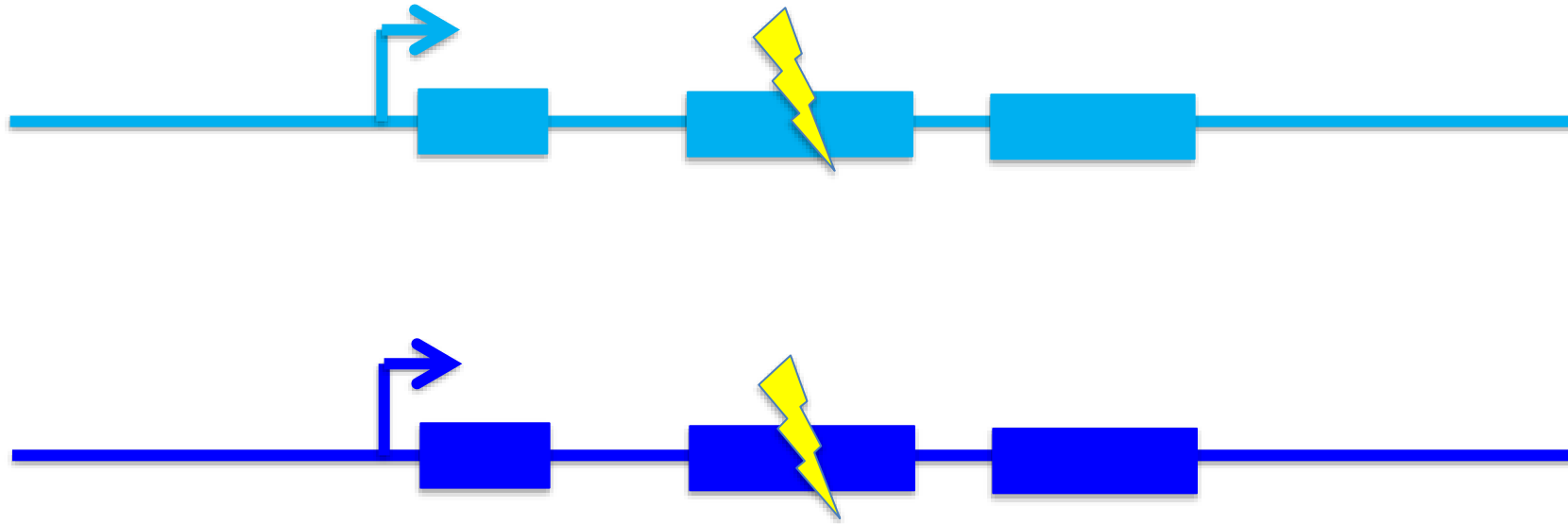
Validación de tratamientos



Limitaciones de las herramientas CRISPR de primera generación

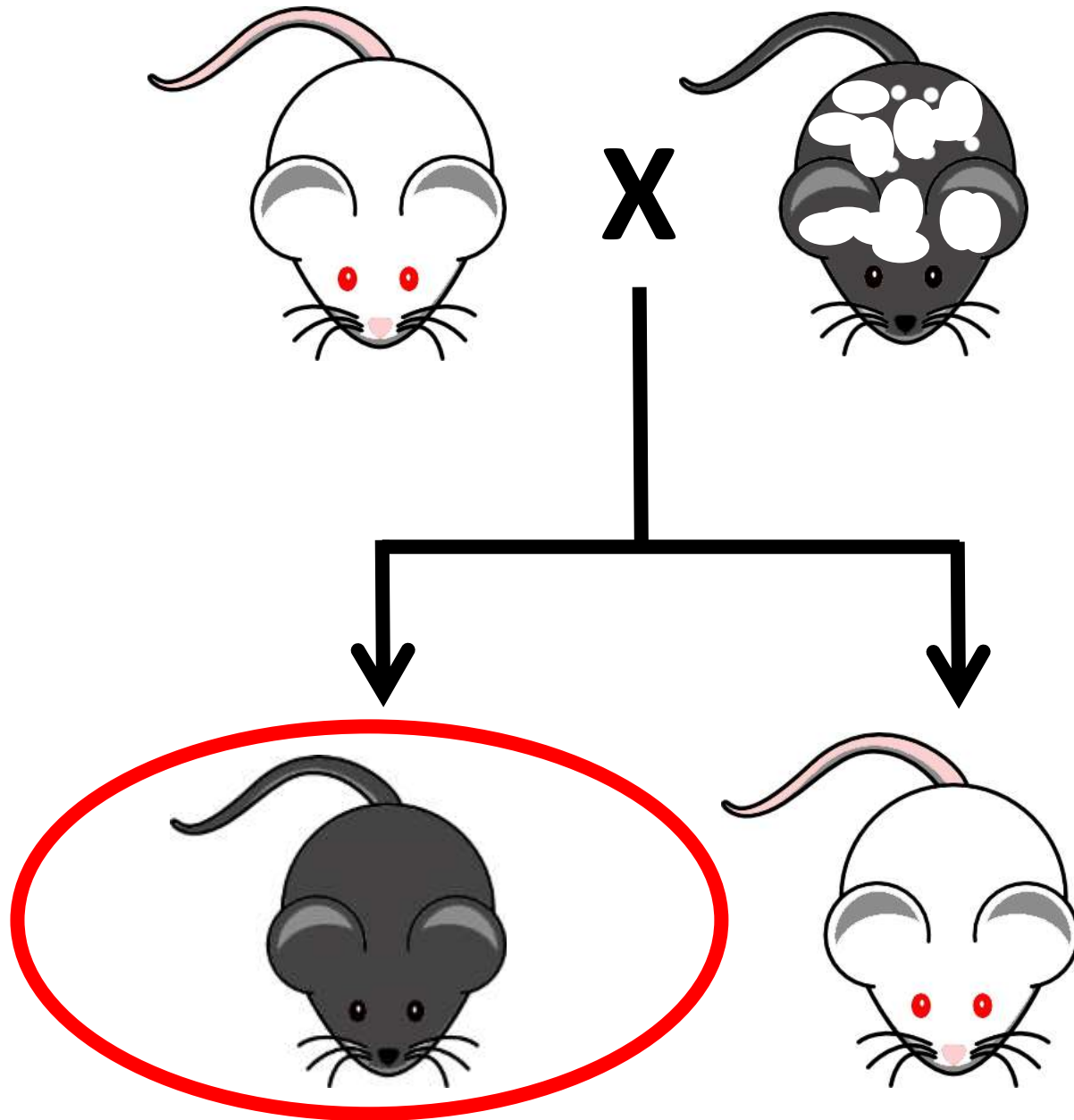


Inactivación de genes parecidos



Mosaicismo







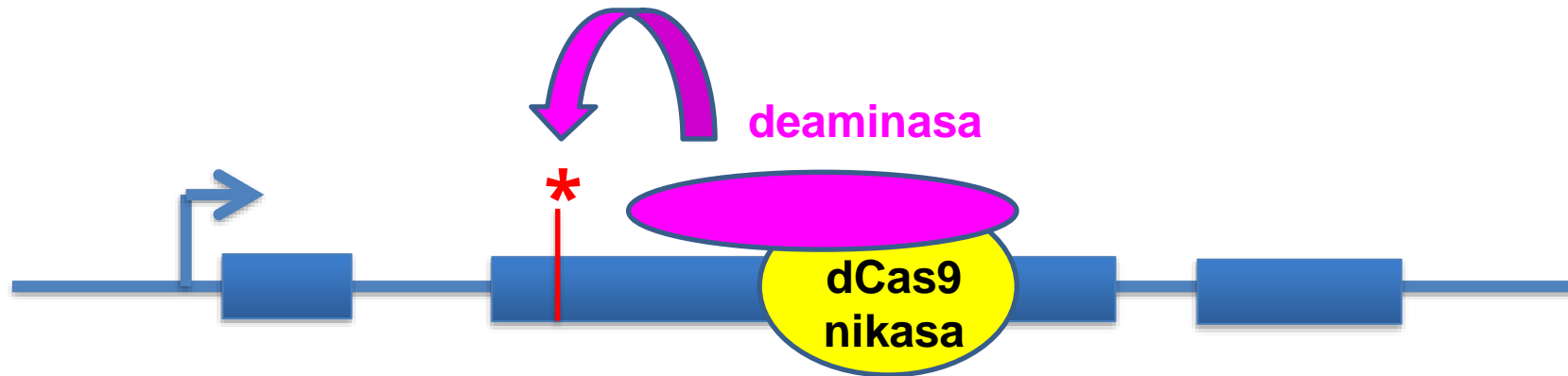
Soluciones a las limitaciones de las herramientas CRISPR de primera generación



A portrait of David R. Liu, a man with short dark hair, wearing a dark blue patterned blazer over a light blue button-down shirt. He is standing in a modern office or lounge area with large windows in the background. The windows show a view of a building and some greenery. In the foreground, there are two orange armchairs. The lighting is soft and professional.

David R. Liu
BROAD Institute

Editores de bases



Segunda generación de herramientas CRISPR

C → T

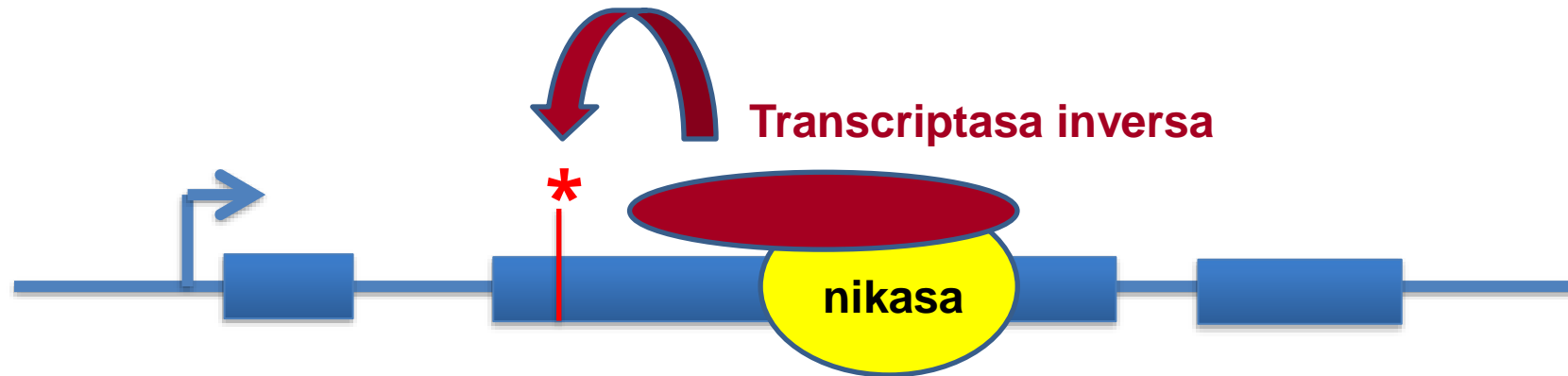
A → G

C → G



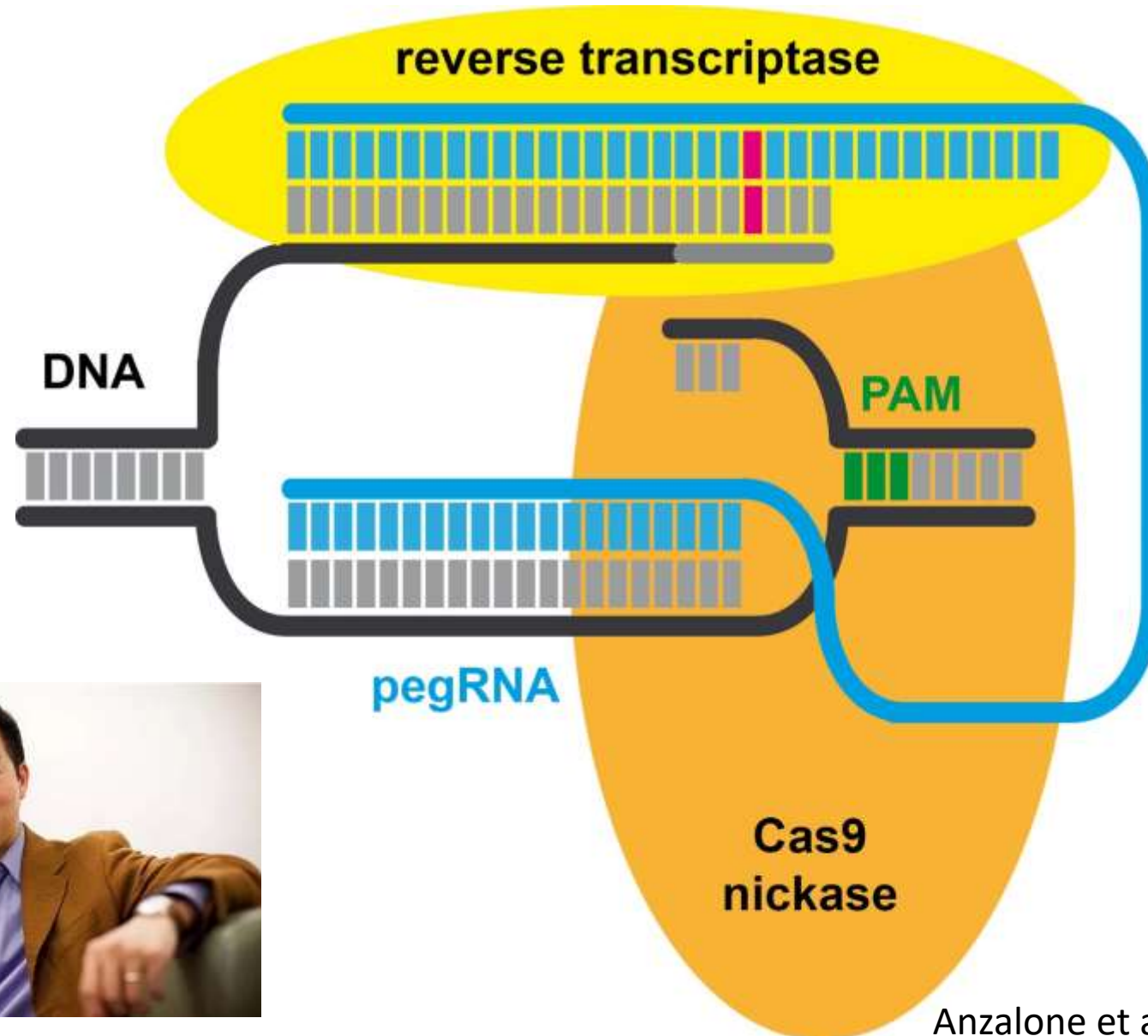
AGGATTAGAGCCGATAGCATACGATCAGTACGAT

Édición de calidad



Tercera generación de herramientas CRISPR

Edición de calidad



David Liu Lab



La CRISPR más precisa hasta la fecha convierte la tijera genética en una navaja suiza

Publicado: 23 octubre 2019 22:30 CEST

IN VIVO

Terapia génica somática

EX VIVO

Direct delivery

Cell-based delivery

Treatment or missing gene.



Treatment or missing gene.

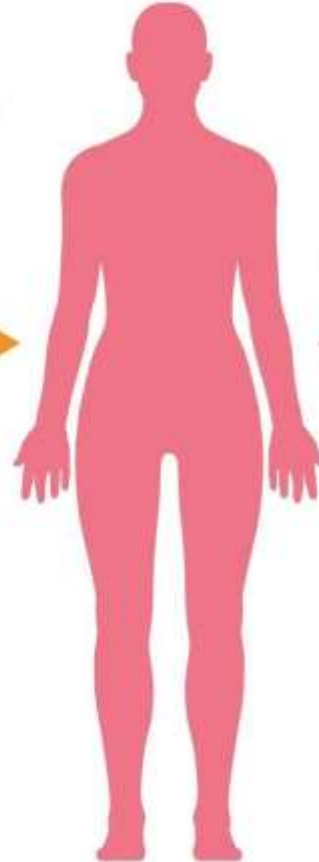
The treatment gene is added to a vector, such as an adeno-assisted virus...



The treatment gene is added to a harmless retrovirus or lentivirus...



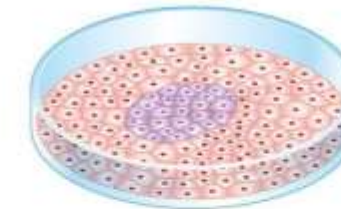
...which is delivered directly to the patient by injection.



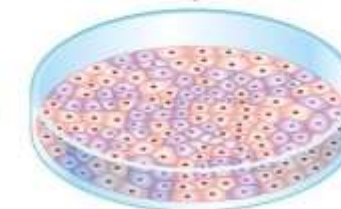
The patient's own stem cells are removed from the body and cultured.



...which, in turn, introduces it to the isolated stem cells.



The stem cells, now containing the treatment gene, are returned to the patient.

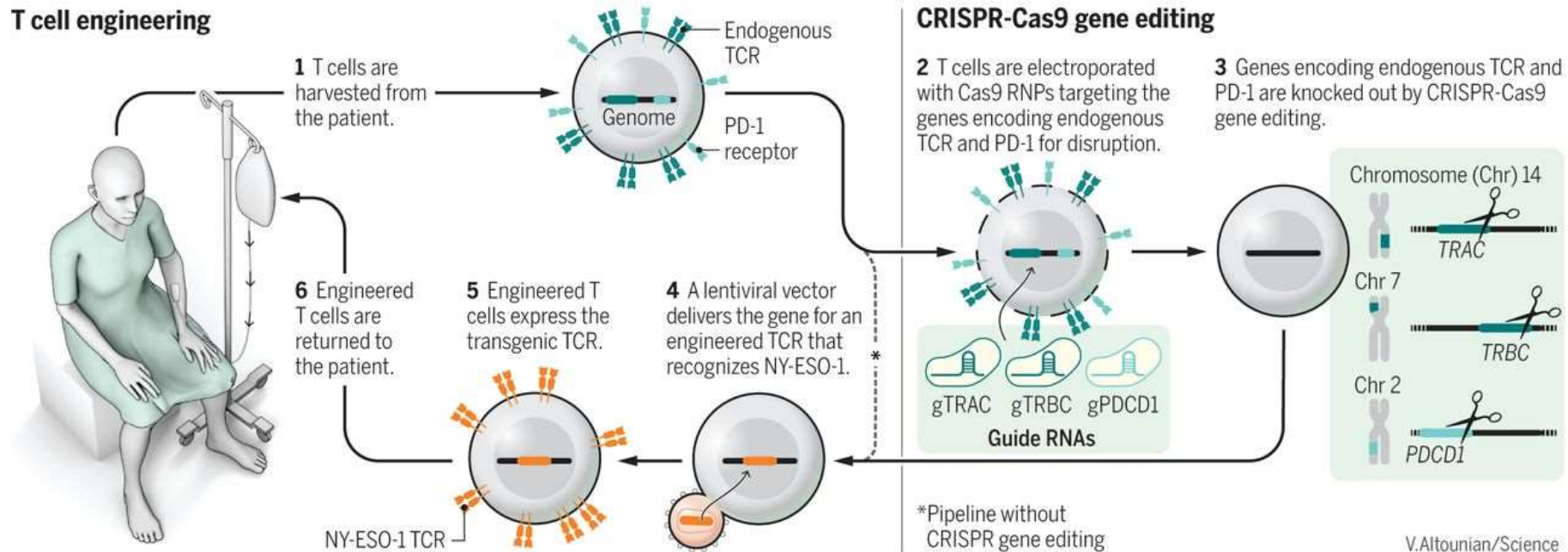


Inmunoterapia del cáncer con CRISPR

Modifying engineered T cells with CRISPR-Cas9 gene editing

Engineered T cells with improved anticancer activity can be generated through the targeted disruption of immunomodulatory genes, such as programmed cell death protein 1 (*PDCD1*, which encodes PD-1), and T cell receptor (TCR) genes (*TRAC* and *TRBC*), using CRISPR-Cas9 delivered as preformed ribonucleoproteins (RNPs). These cells are then modified to express an engineered TCR that recognizes cancer-testis antigen 1 (NY-ESO-1) expressed by cancer cells.

T cell engineering



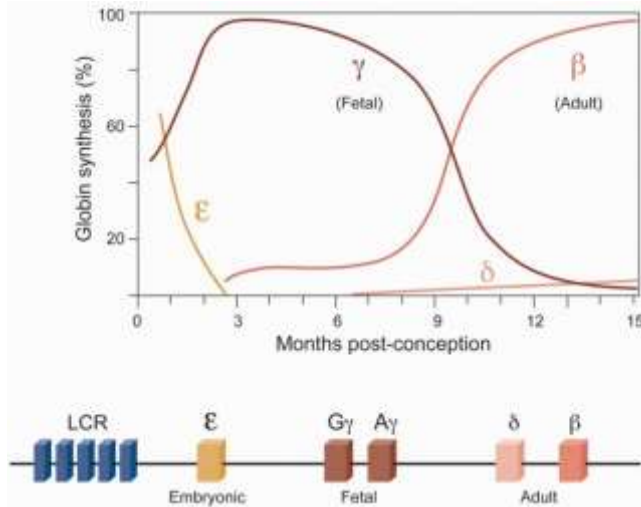


**Tratada su leucemia
linfoblástica aguda de
células T con editores
de bases y con
tecnología CAR-T**

Mayo 2022

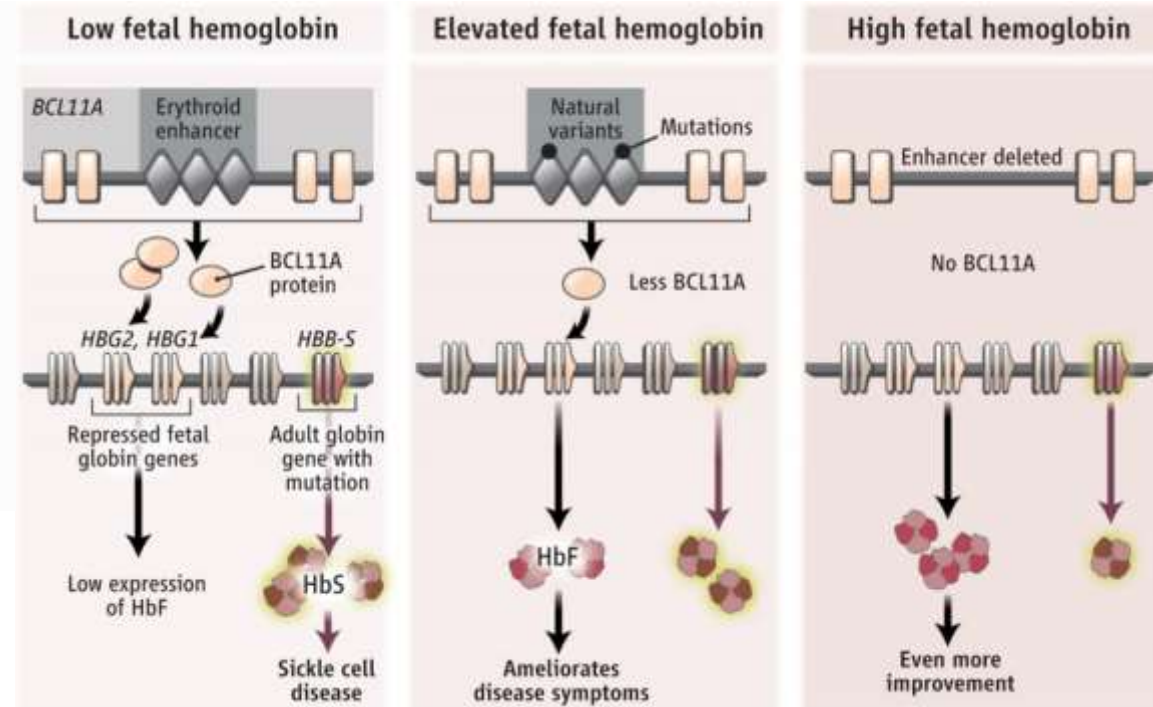
Alyssa

Curar la anemia falciforme con terapia génica CRISPR



Anemia falciforme

1:5.000/10.000



Bauer Lab (Dana Farber-Boston Children's)

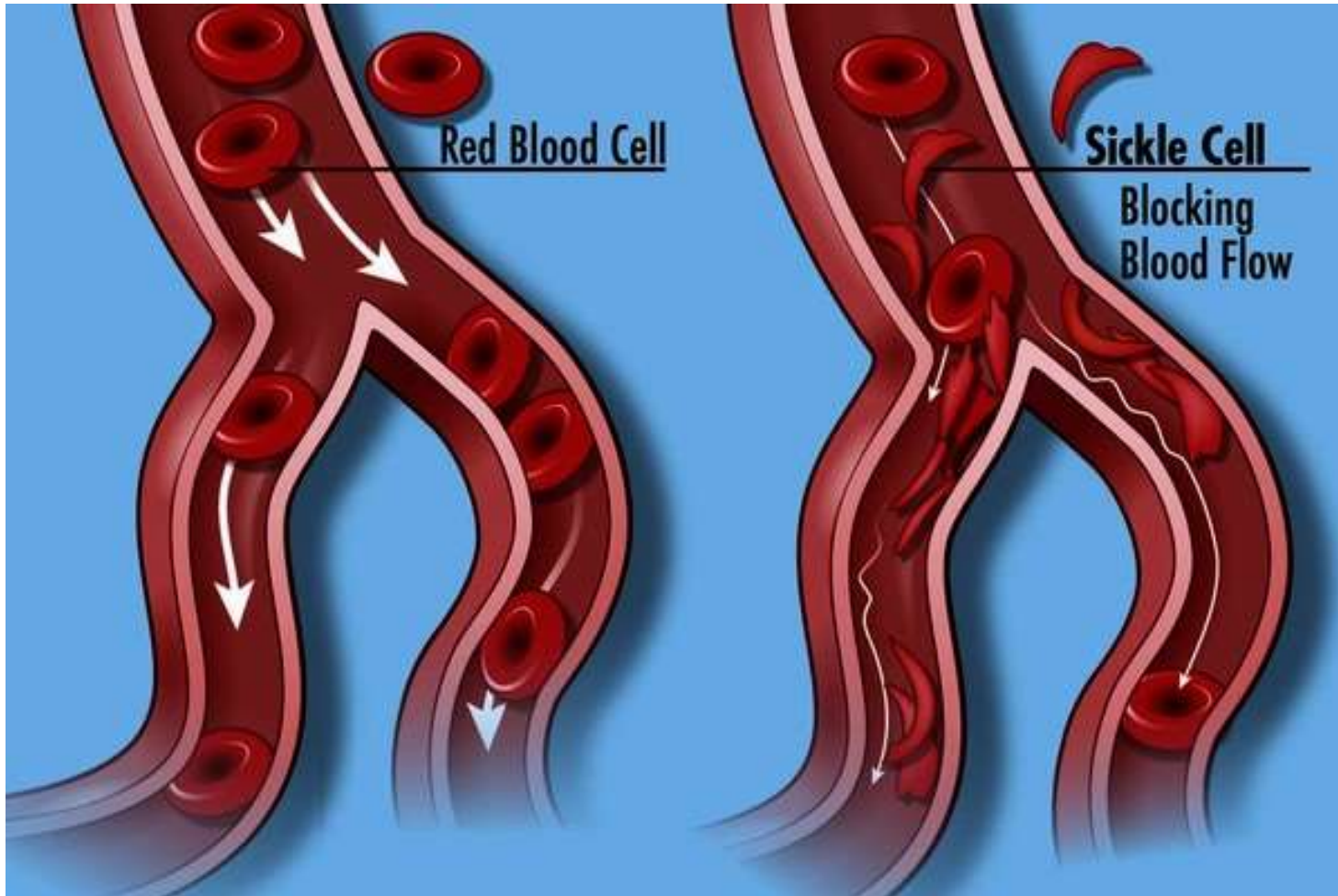


Los primeros ensayos clínicos **ex-vivo**
Con CRISPR aprobado en Europa

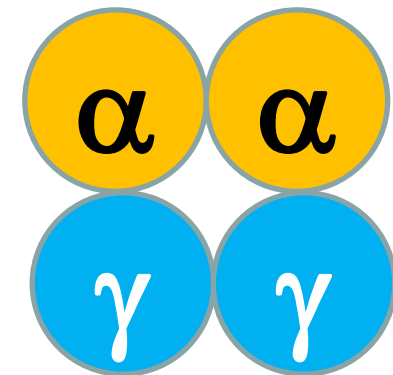
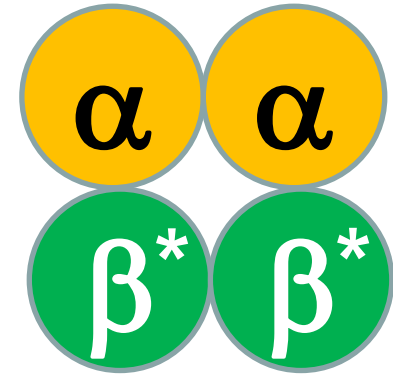
November 2019

Glóbulo rojo normal

Glóbulo rojo en anemia falciforme



Hemoglobina en anemia falciforme

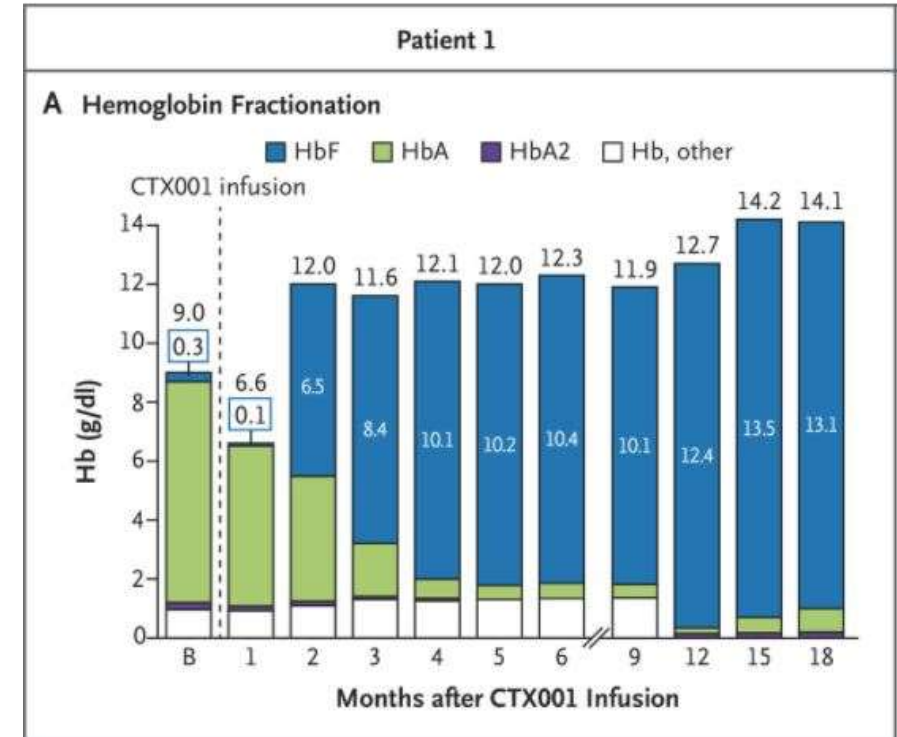


Hemoglobina curada con CRISPR

CRISPR para tratar ANEMIA FALCIFORME



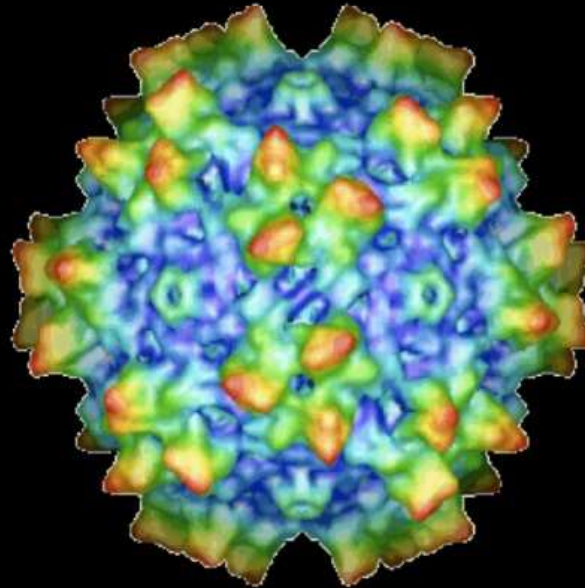
Victoria Gray (tratada 2 julio 2019)



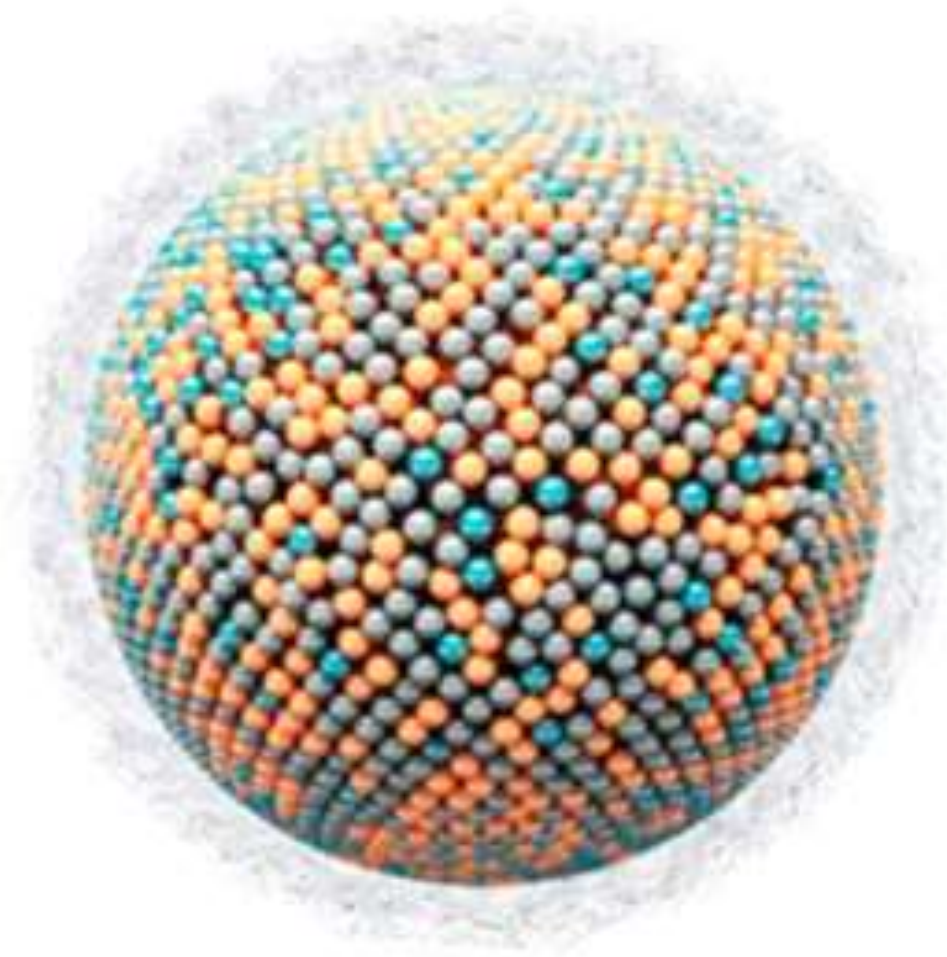
Frangoul *et al.* NEJM (5 December 2020)

Primer paciente curado en EE.UU. (2020)

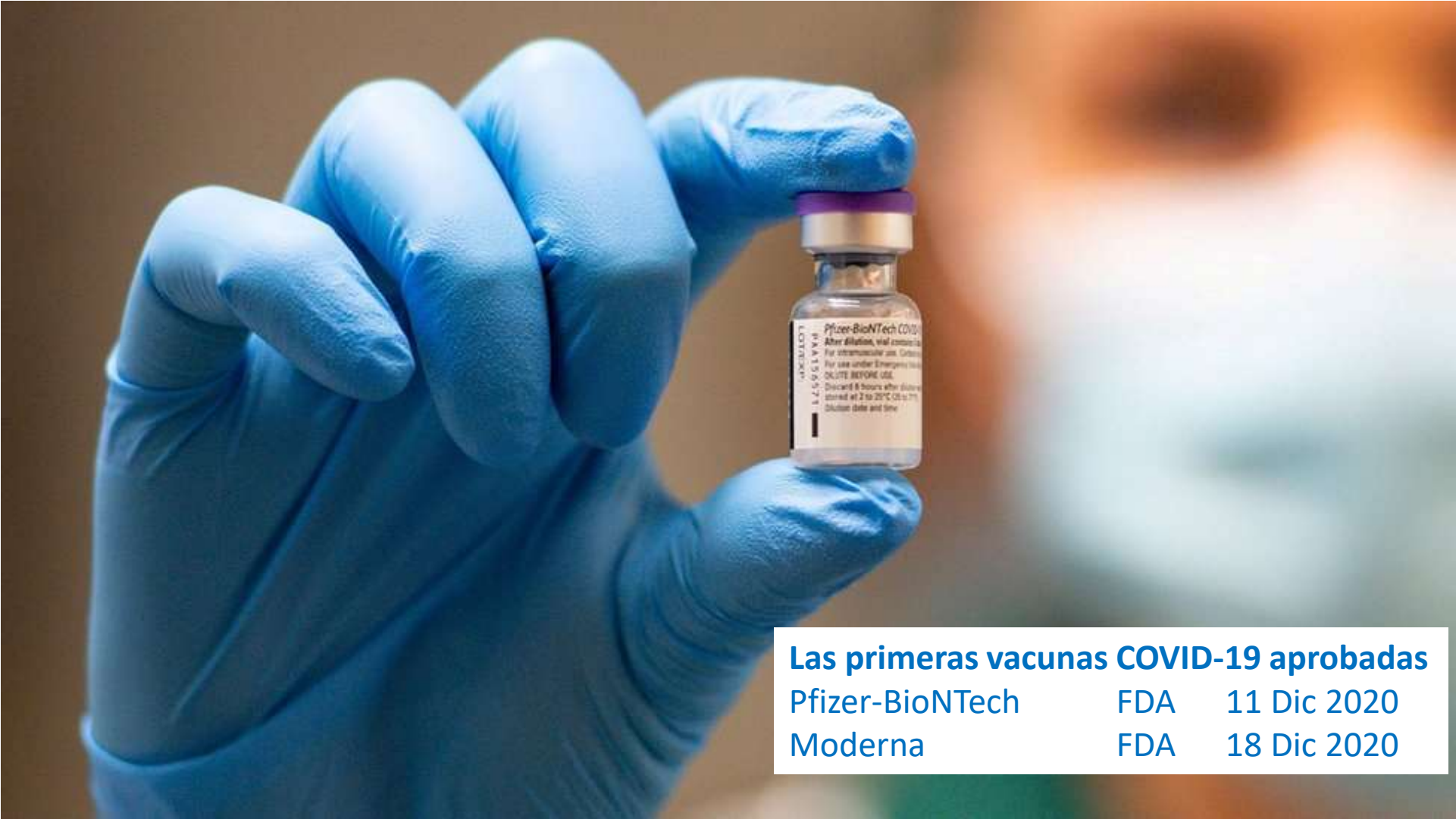
**La mayoría de ensayos clínicos CRISPR
usan virus adenoasociados para administrarlas**



AAV



Nanopartículas lipídicas

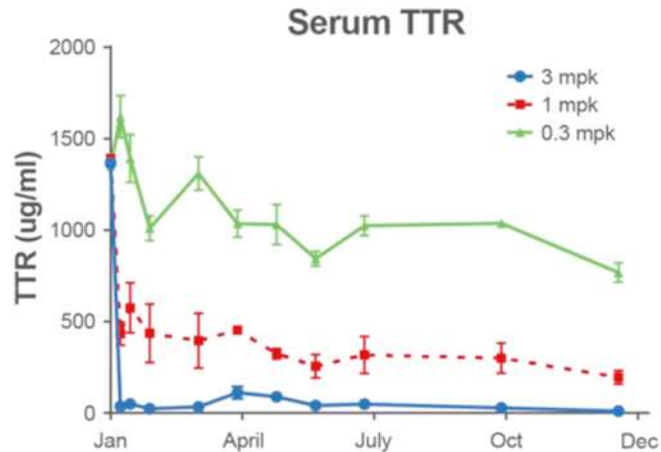
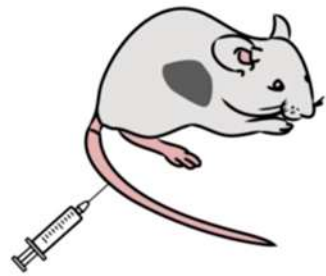
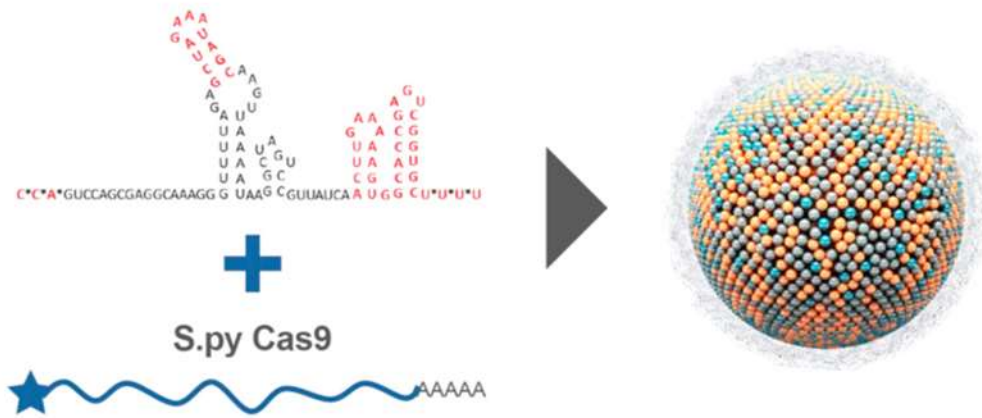


Las primeras vacunas COVID-19 aprobadas

Pfizer-BioNTech	FDA	11 Dic 2020
Moderna	FDA	18 Dic 2020

Amiloidosis congénita por transtiretina (ATTR) NANOTECNOLOGÍA - Nanopartículas

1-9:1.000.000



Finn et al. *Cell Reports* 2018 22, 2227-2235 DOI: (10.1016/j.celrep.2018.02.014)
Copyright © 2018 Intellia Therapeutics, Inc.



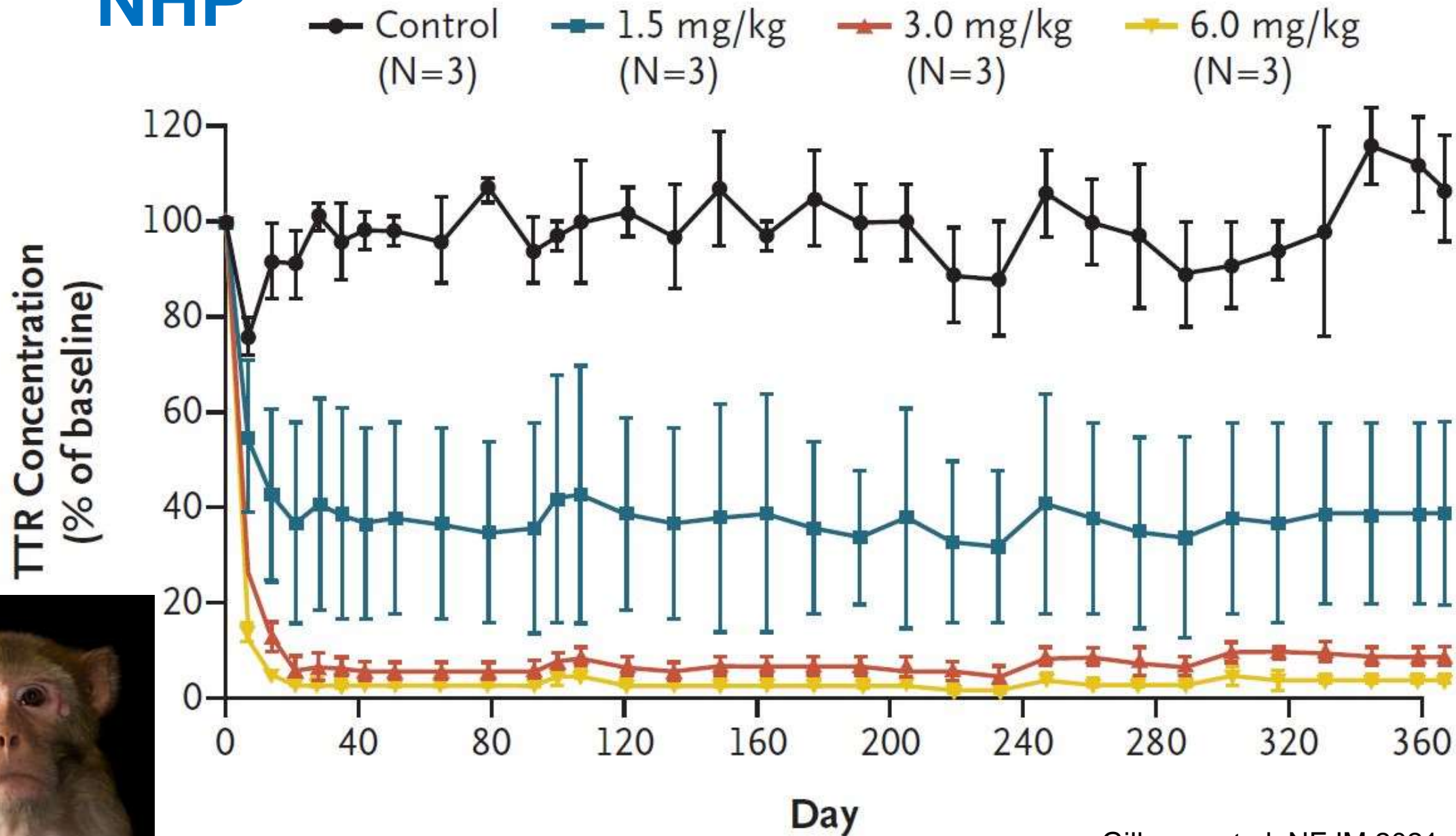
Investors & Media
Press Releases
Events & Presentations
Corporate Governance

Oct 19, 2020

NTLA-2001: First single-course therapy that potentially halts and reverses ATTR

On track to dose first patient by year-end with a systemically delivered CRISPR/Cas9-based therapy

October 2020

A**NHP**

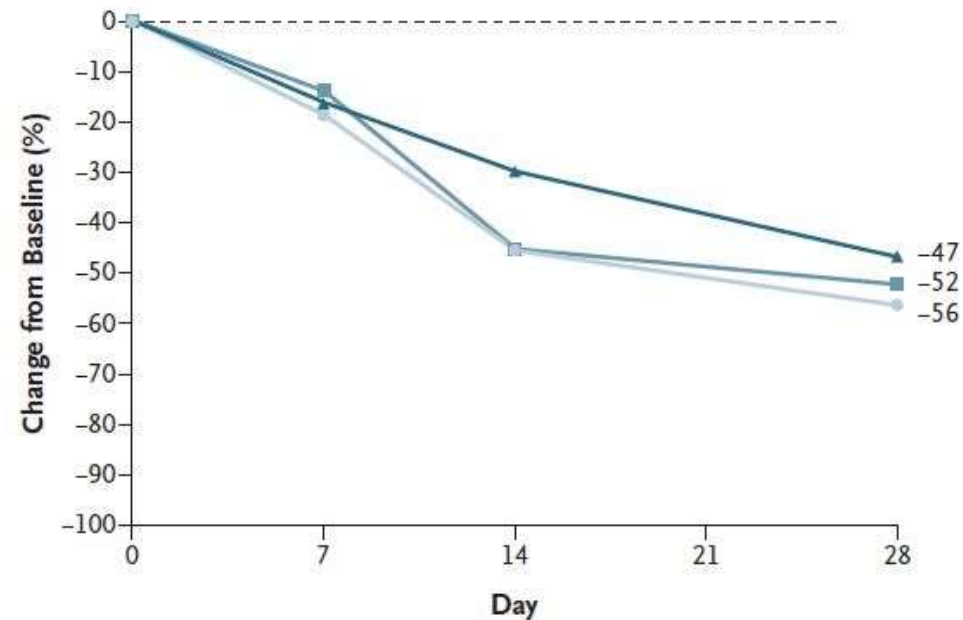


Paddy Doherty

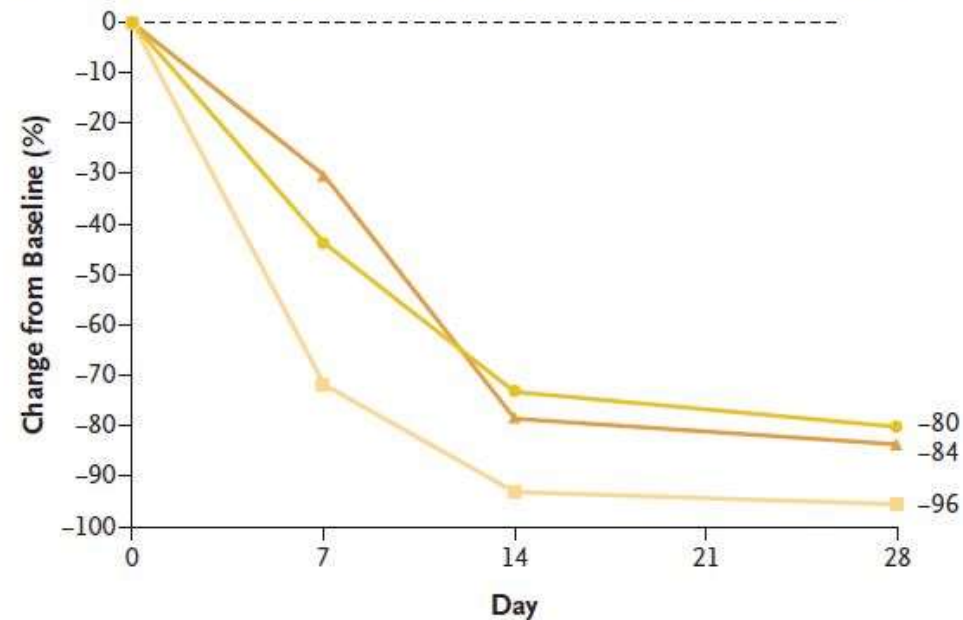
CRISPR para tratar la amiloidosis por transtiretina congénita

Gillmore et al. NEJM 2021

A Change in Serum TTR Concentration in Patients Who Received 0.1 mg/kg



B Change in Serum TTR Concentration in Patients Who Received 0.3 mg/kg





Cas9

Streptococcus pyogenes
Staphylococcus aureus

Cas9: Bang Wong, Broad Institute of Harvard and MIT, Cambridge, MA



Matthew Porteus **2019 *Nature Med.***

Las CRISPR actuales derivan de bacterias patógenas

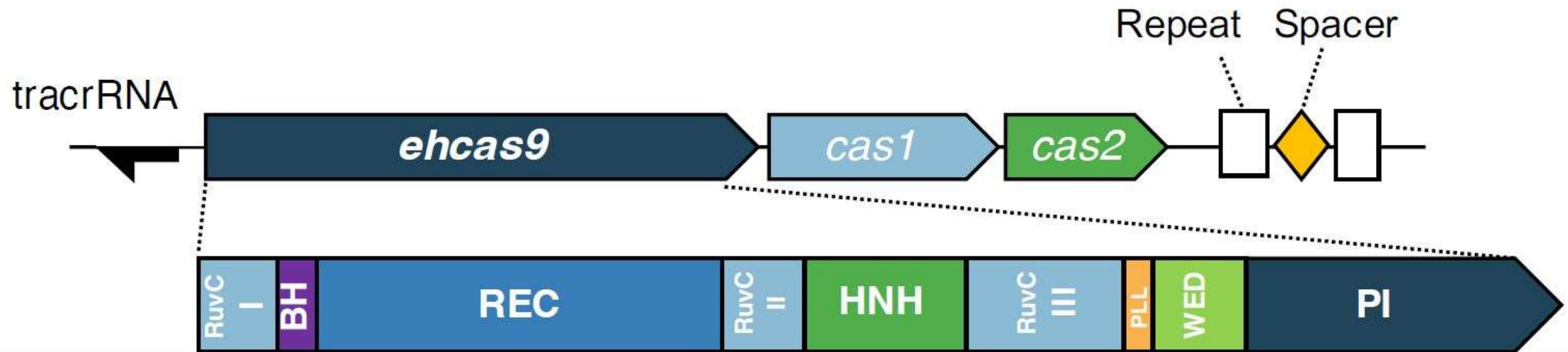


RESEARCH ARTICLE



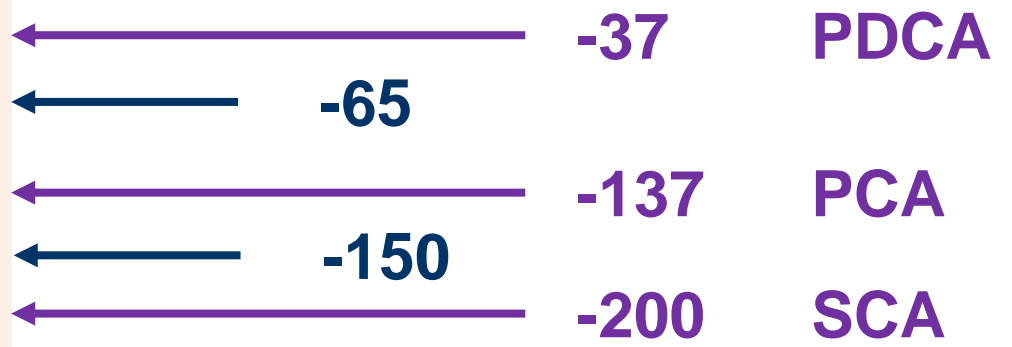
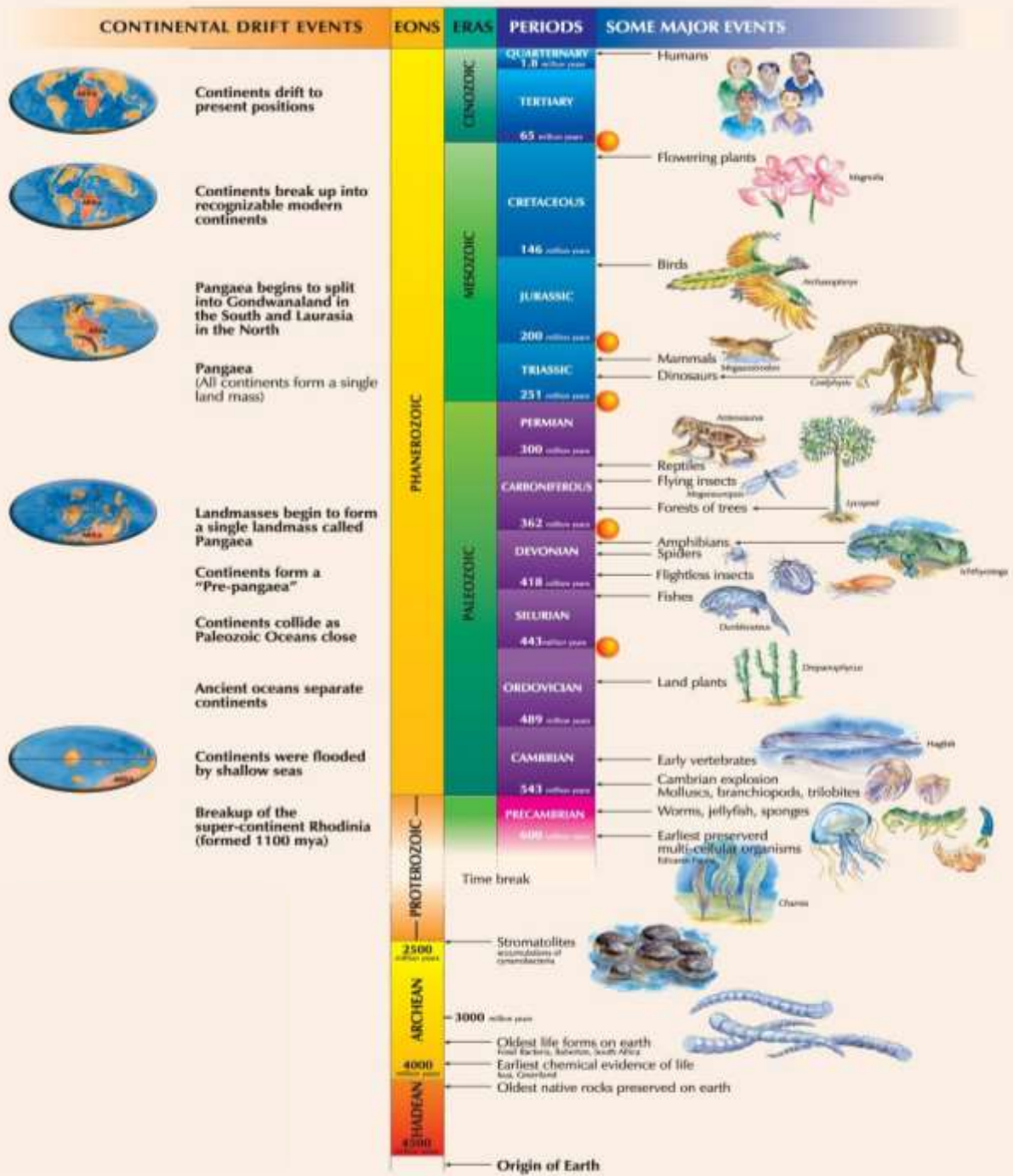
Identification of the EH CRISPR-Cas9 system on a metagenome and its application to genome engineering

Belen Esquerra-Ruvira¹ | Ignacio Baquedano¹ | Raul Ruiz¹ |
Almudena Fernandez^{2,3} | Lluís Montoliu^{2,3} | Francisco J. M. Mojica^{1,4}



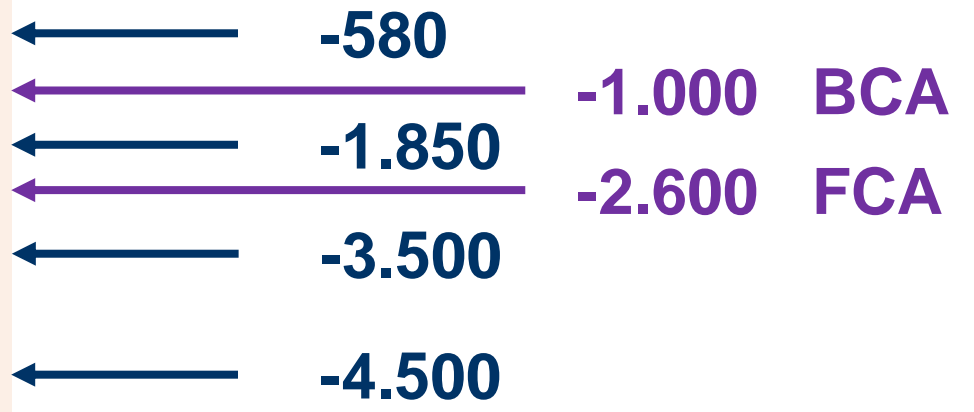


¿Cómo serían las CRISPR-Cas ancestrales?



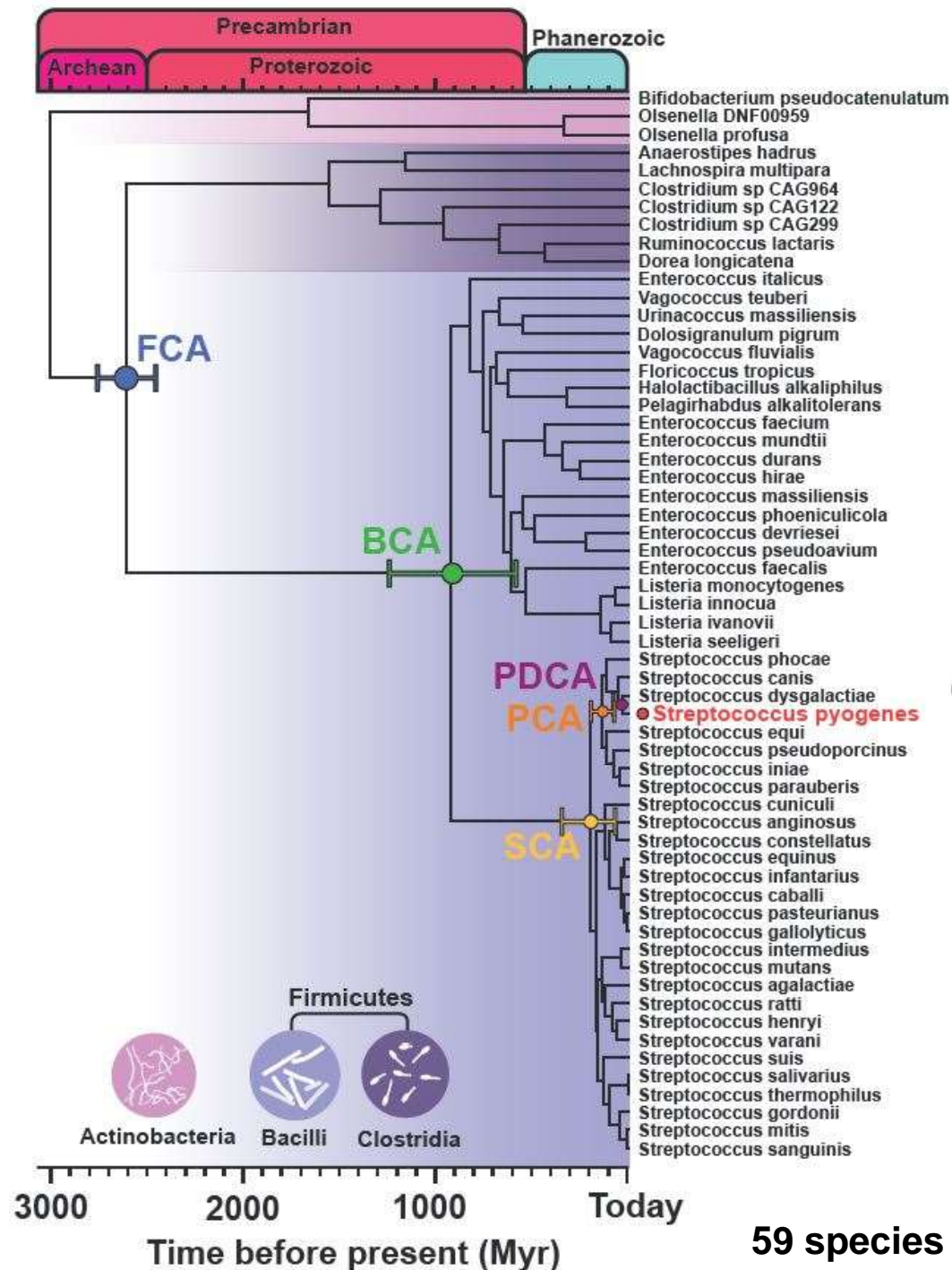
Resucitar Cas ancestrales

**Raúl Pérez- Jiménez Lab
CIC nanoGUNE**



My

Raúl Pérez-Jiménez Lab
CIC nanoGUNE

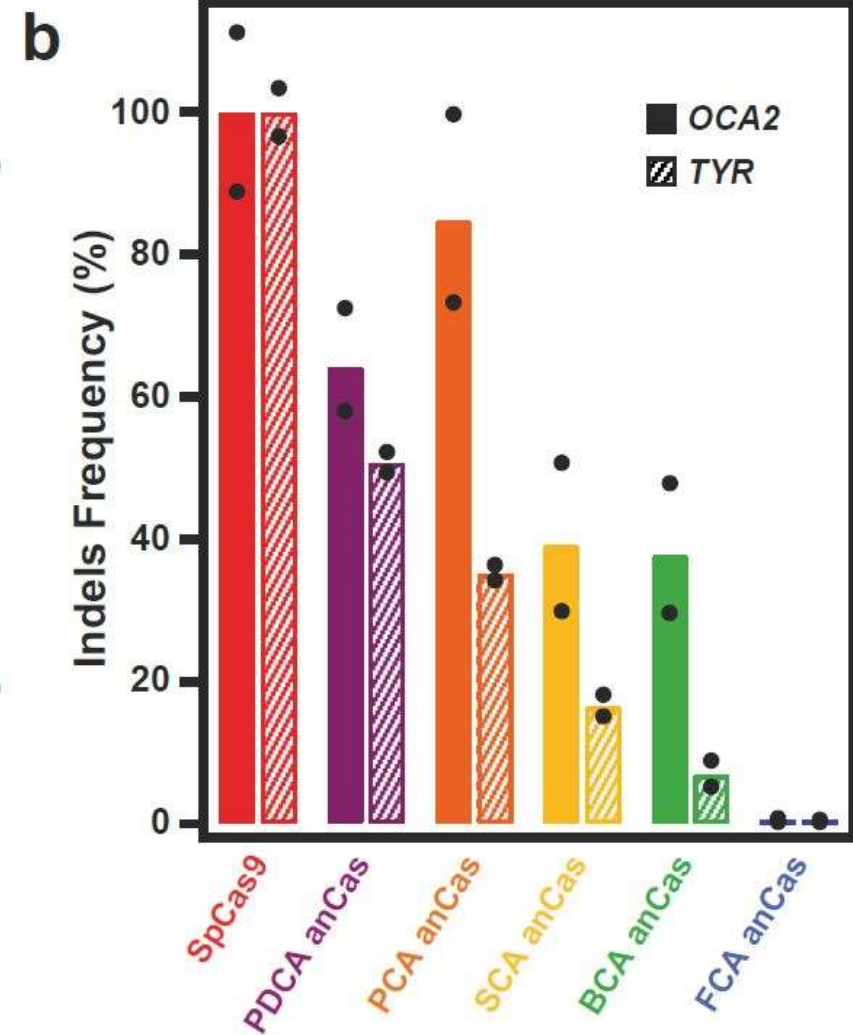
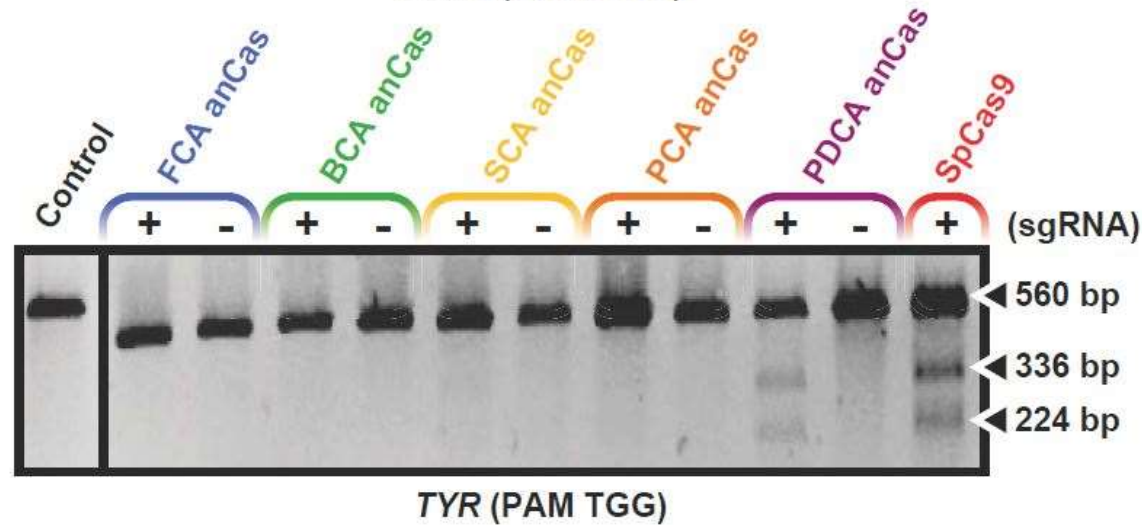
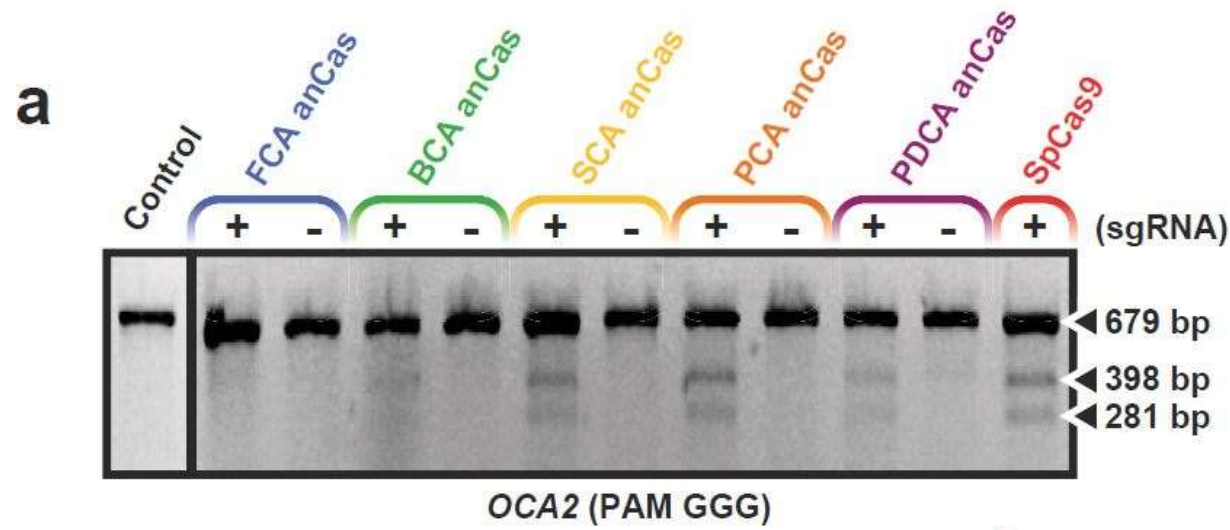


Reconstrucción de
secuencias Cas
ancestrales
mediante un
procedimiento
estadístico y
superordenadores

53-96% similitud

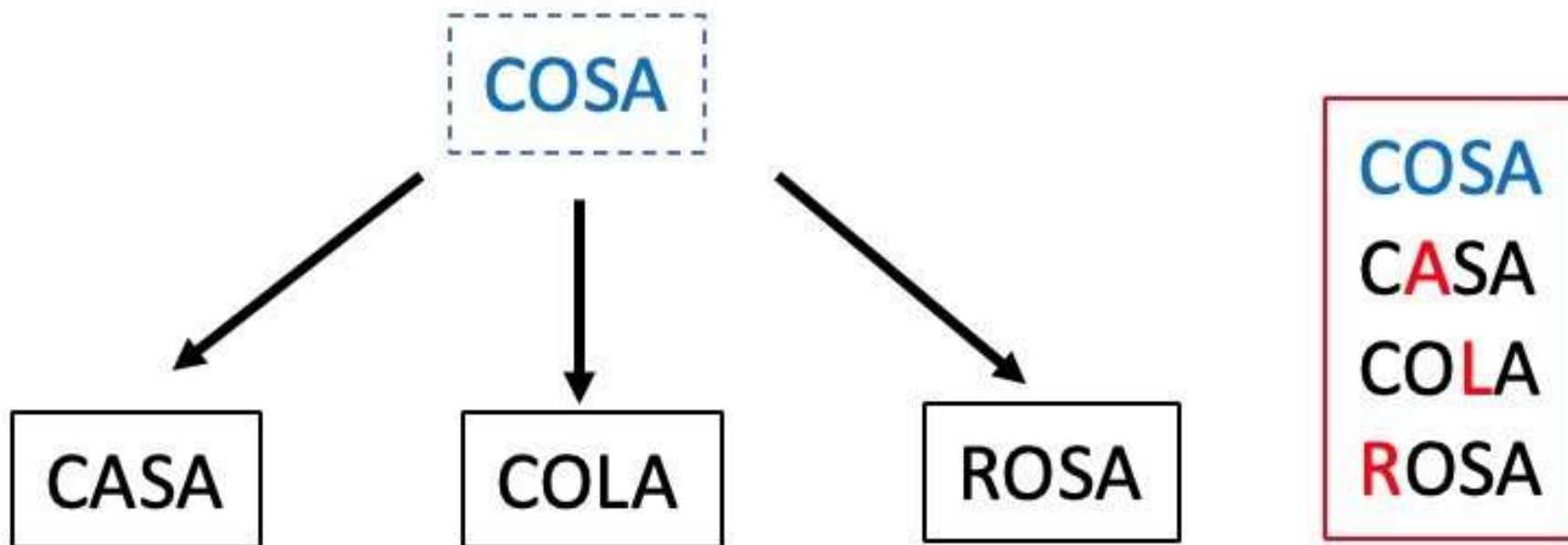
Las Cas ancestrales funcionan como editores génicos en células humanas

Alonso-Lerma et al. 2023 Nature Microbiology



c

d



CRISPRpedia



CRISPRpedia is a free, textbook-style resource that explains and illustrates all things CRISPR.

<https://innovativegenomics.org/crisprpedia/>