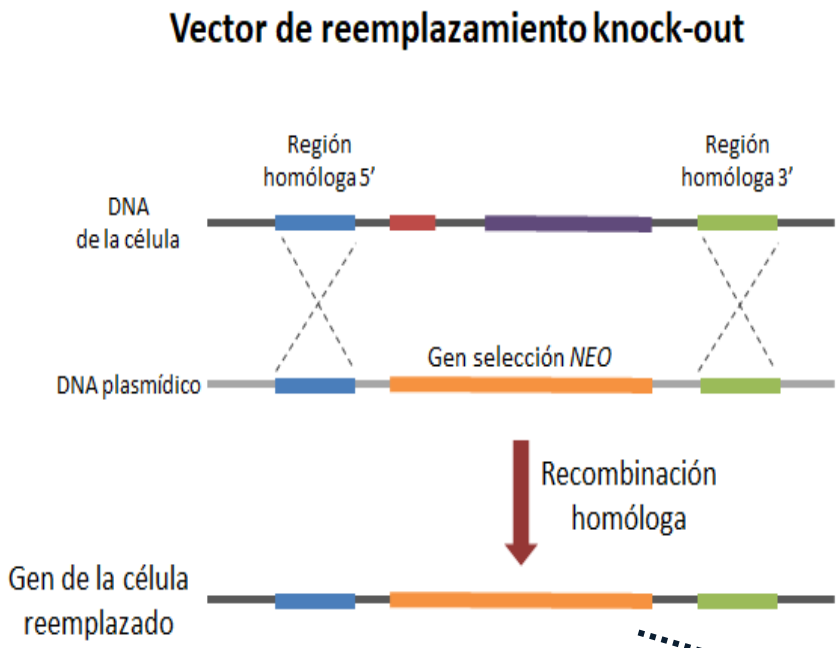
JUGANDO CON EL GENOMA:

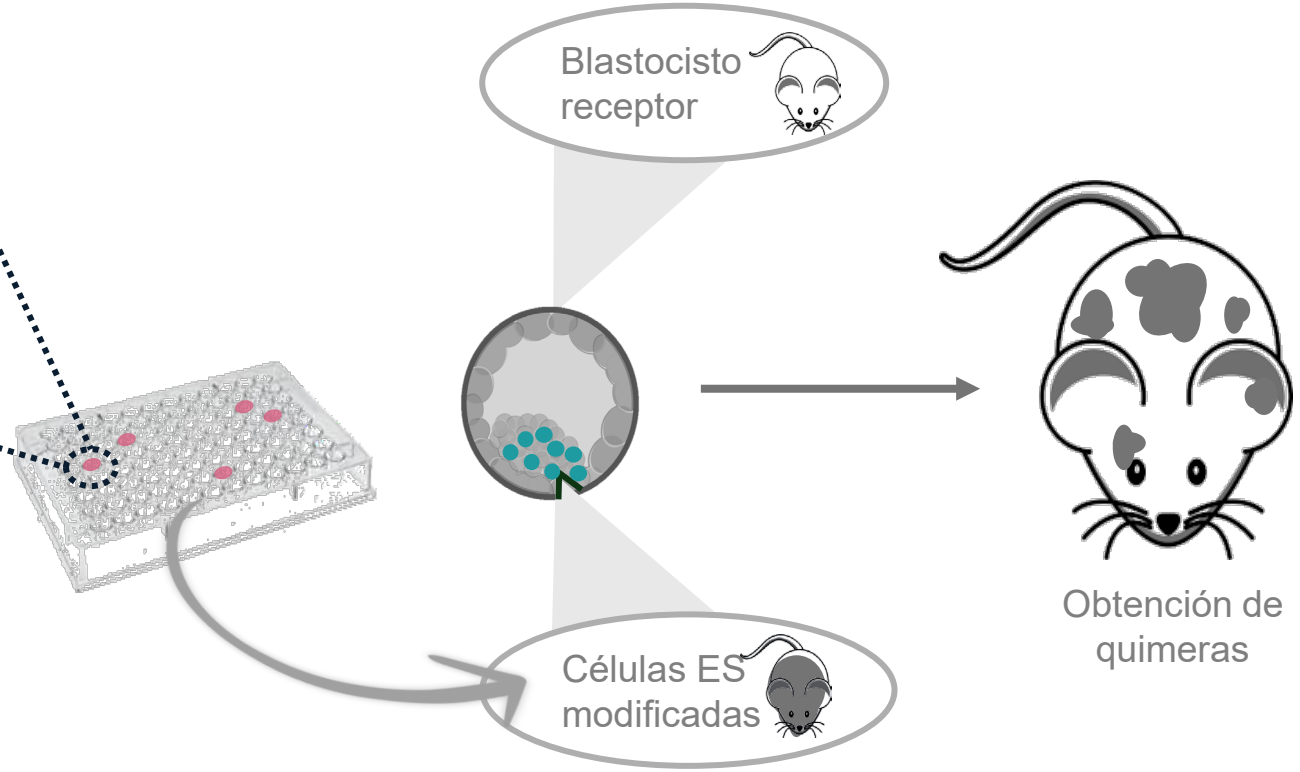
CRISPR/CAS9

ANTES DE CRISPR: GENE TARGETING

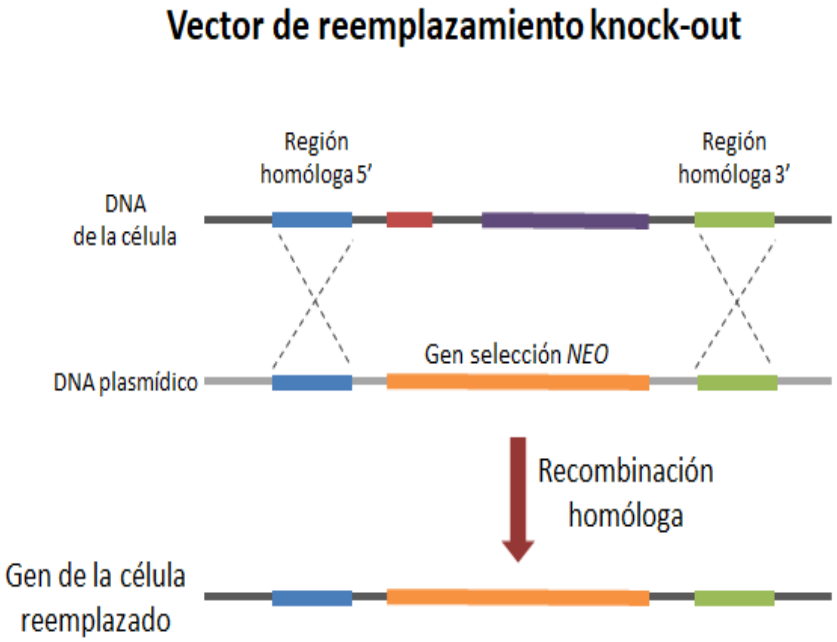


- Limitado a ciertas especies (no humanos)
- Limitado a la frecuencia de RH

- > 1 año para obtener quimeras
- Emplea genes resistencia
- Frecuencia 1/120 – 1/240 clones **(0,5%)**



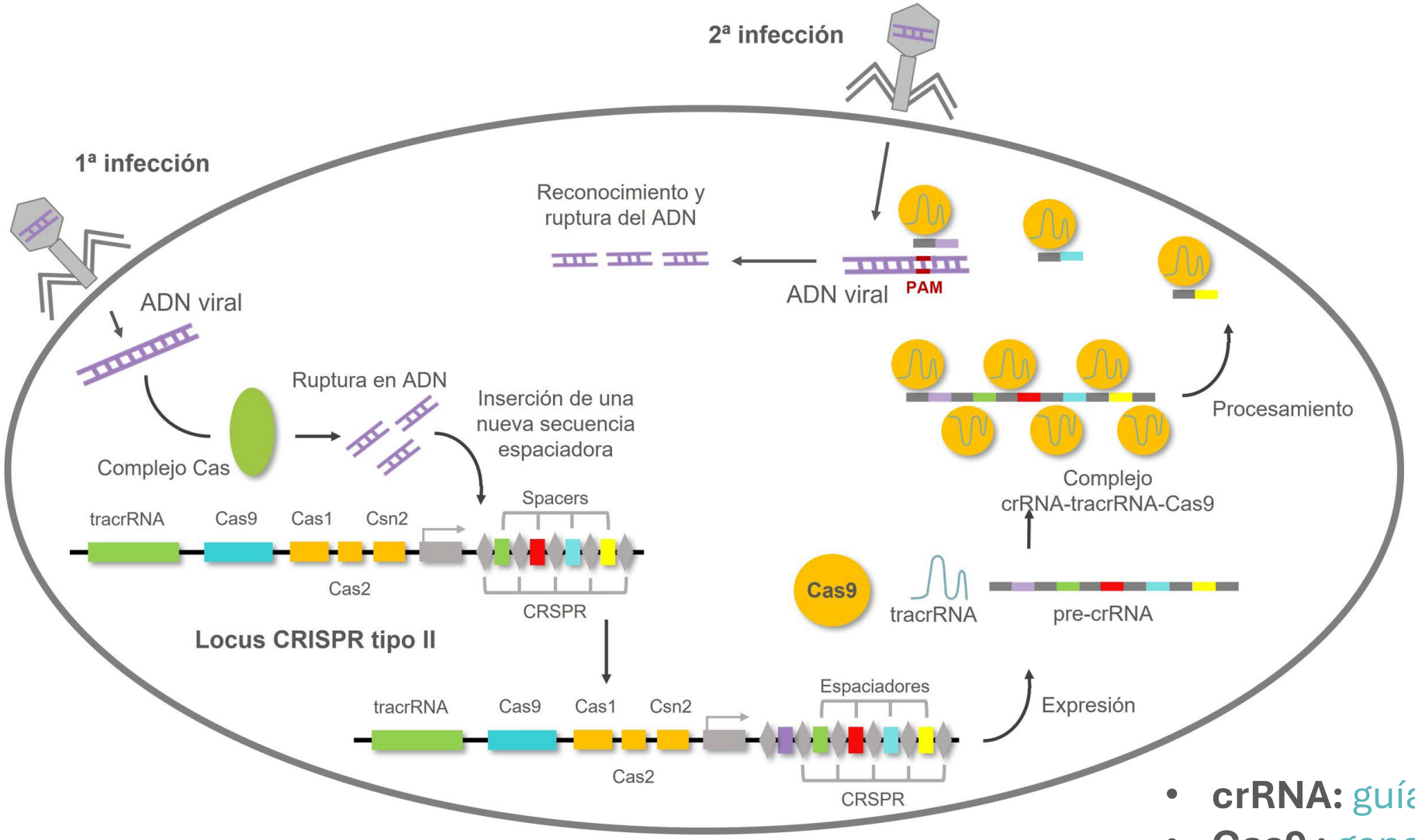
ANTES DE CRISPR: GENE TARGETING



¿CÓMO INCREMENTAR LA FRECUENCIA DE MODIFICACIÓN GENICA?

CORTES DIRIGIDOS EN EL GENOMA (DSB)

EL SISTEMA CRISPR/CAS9



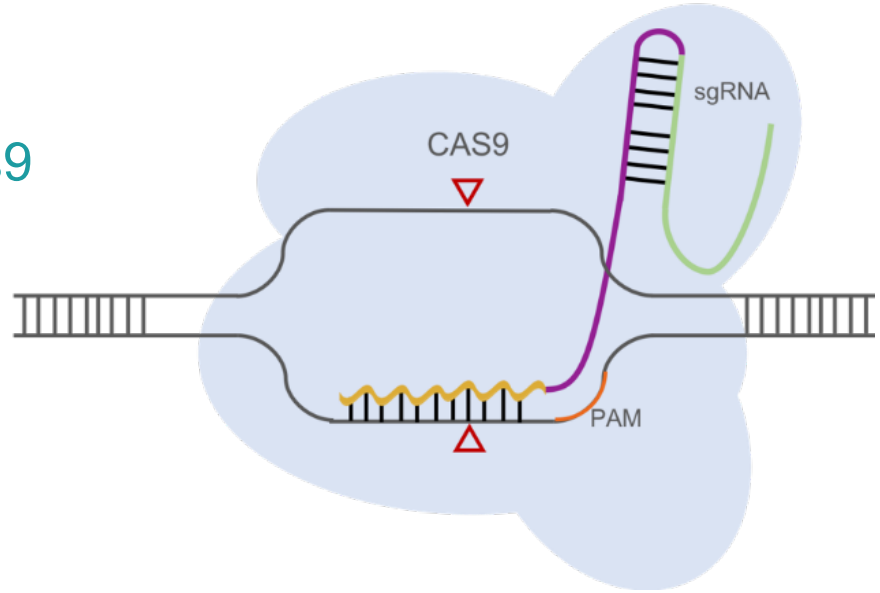
¿NUEVA NUCLEASA EDICIÓN GENÓMICA?

- **crRNA:** guía a Cas9
- **Cas9 :** genera DSB

EL SISTEMA CRISPR/CAS9: APLICACIÓN

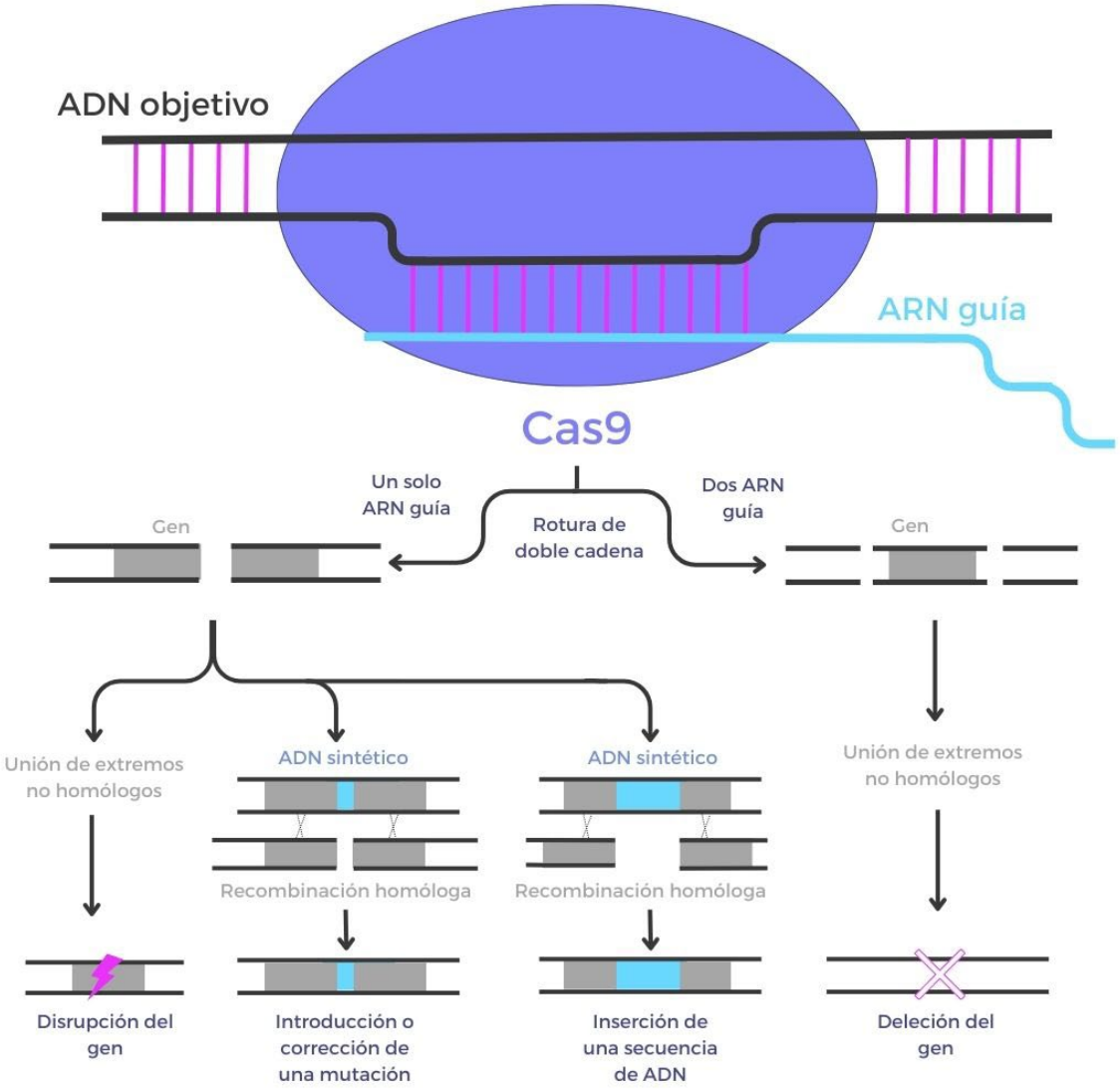
2013: Desarrollo sistema de edición CRISPR/Cas9

E. Charpentier y J. Dounda



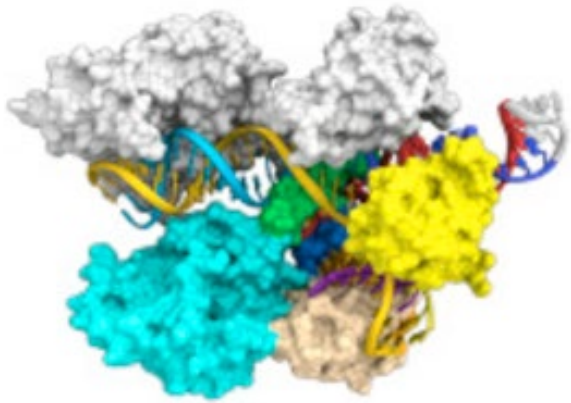
- Dominio de unión al DNA → Single guide RNA (sgRNA)
- Dominio nucleasa → Nucleasa Cas9
- Motivo PAM (5'-NGG-3')

EL SISTEMA CRISPR/CAS9: APLICACIÓN



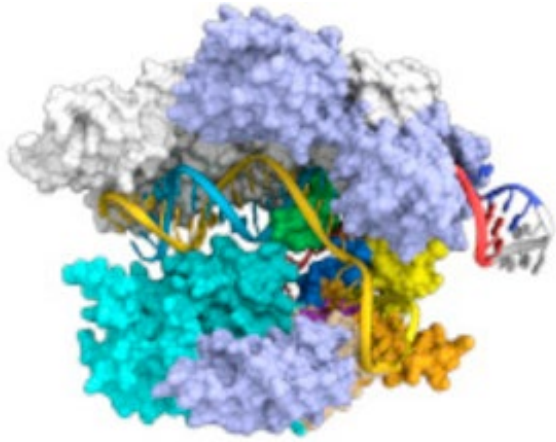
CRISPR/CAS9: NUEVAS PROTEÍNAS CAS

SpCas9
Streptococcus pyogenes



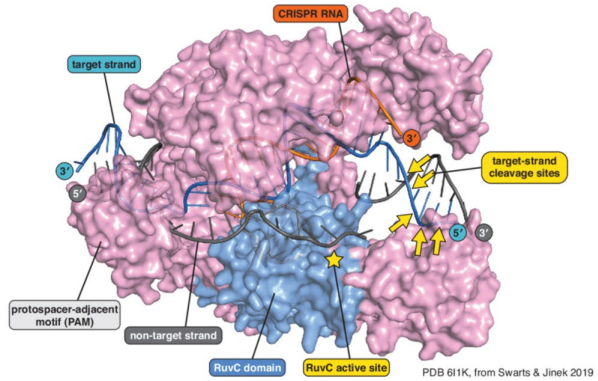
- 163 kDa
- PAM: “NGG”
- Deja extremos romos

SaCas9
Staphylococcus aureus



- 130 kDa
- PAM: “NNGRR(T)”
- Deja extremos romos

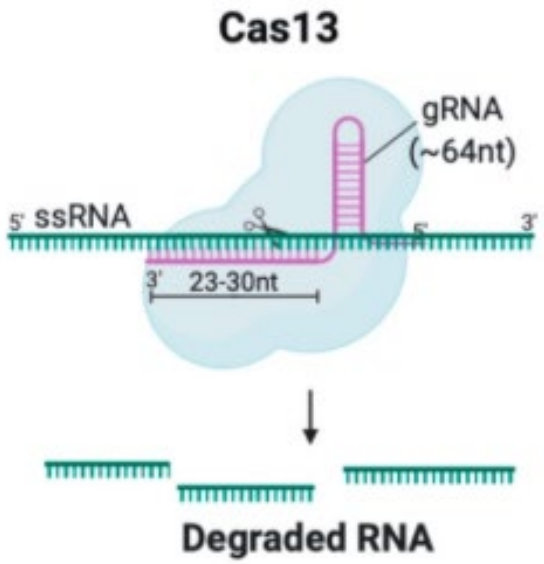
Cas12a (Cpf1)
Acidaminococcus



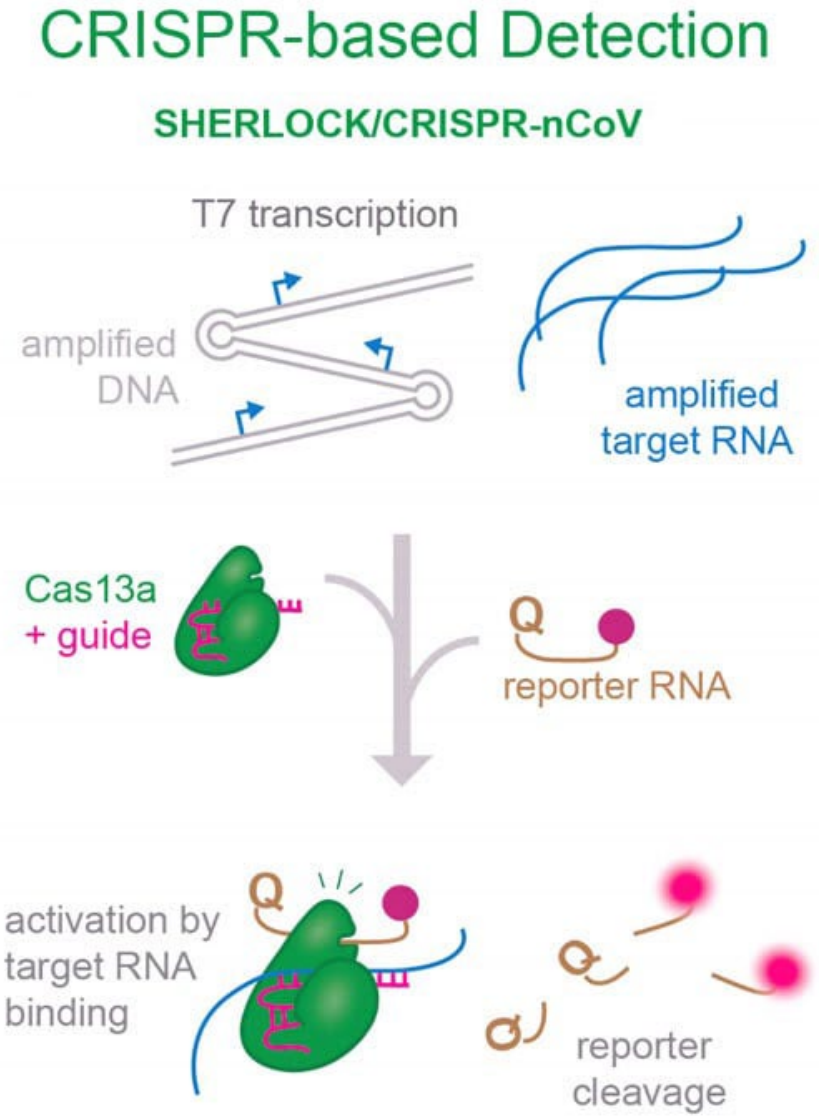
- 145-185 kDa
- PAM: “TTTV”
- Deja extremos protuyentes

CRISPR/CAS9: NUEVAS PROTEÍNAS CAS

Edición del RNA: actividad colateral inespecífica

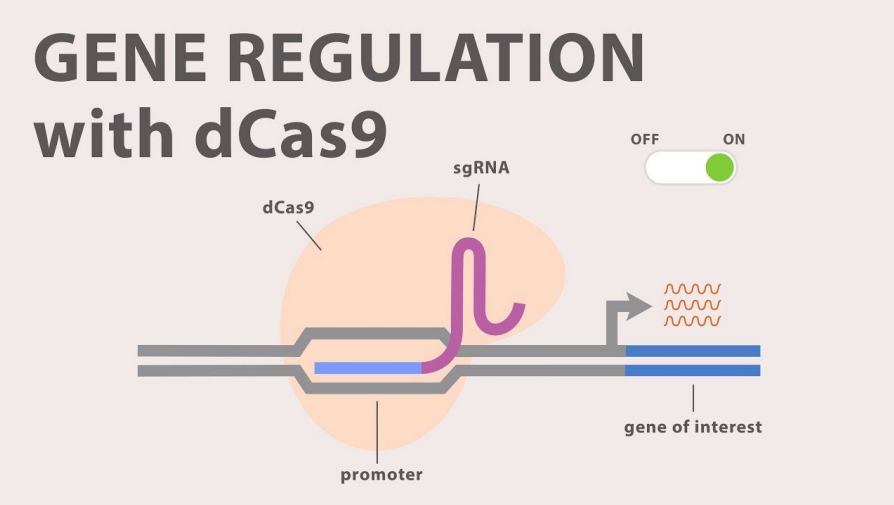


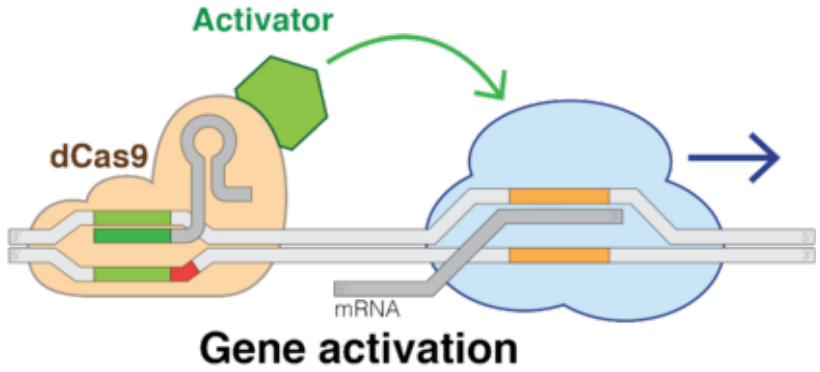
- Gene knockdown
- Terapia antiviral
- Detección RNA viral (SARS-Cov-2)

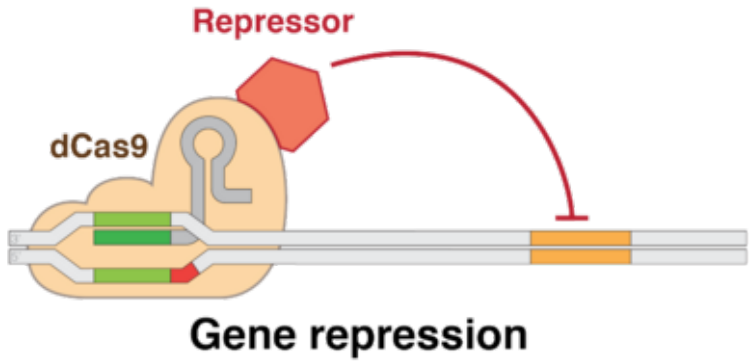


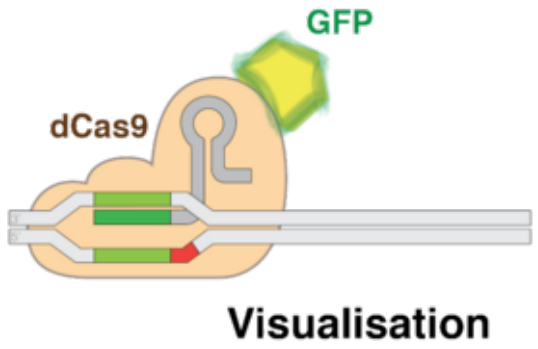
NUEVAS VARIANTES: "THE WORKING DEAD"

- **Dead Cas9:** Cas9 sin actividad nucleasa





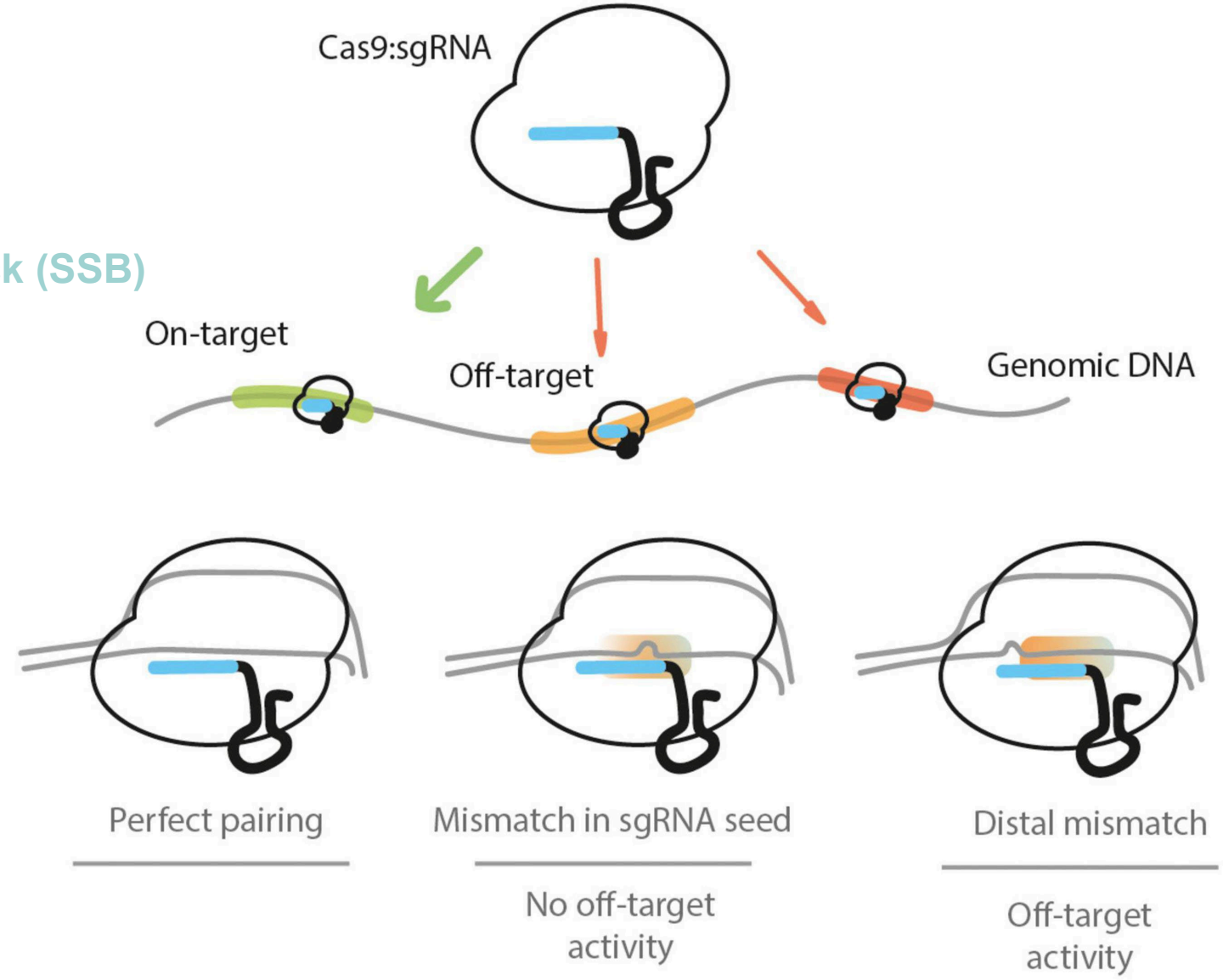




LOS OFF-TARGETS: EL PROBLEMA DEL DSB

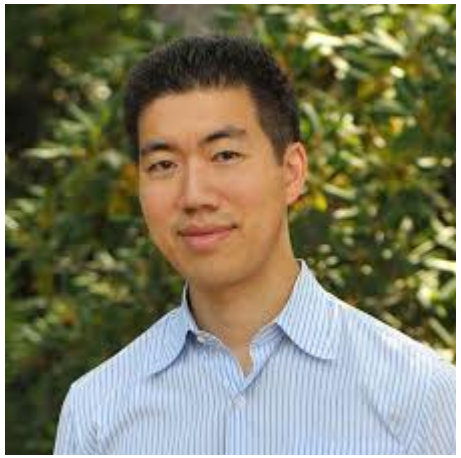
MUTAGÉNESIS

DSB >>>>>>>> Nick (SSB)

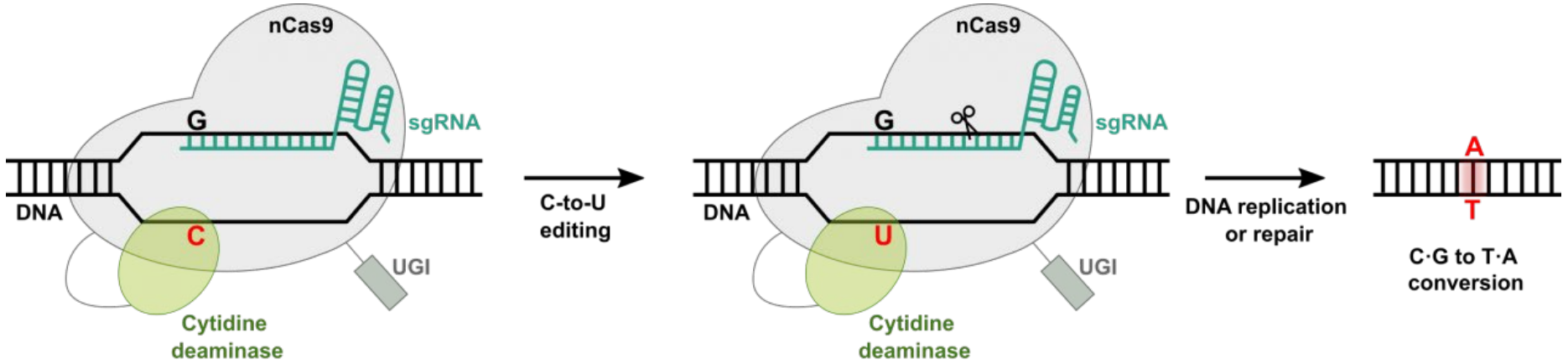


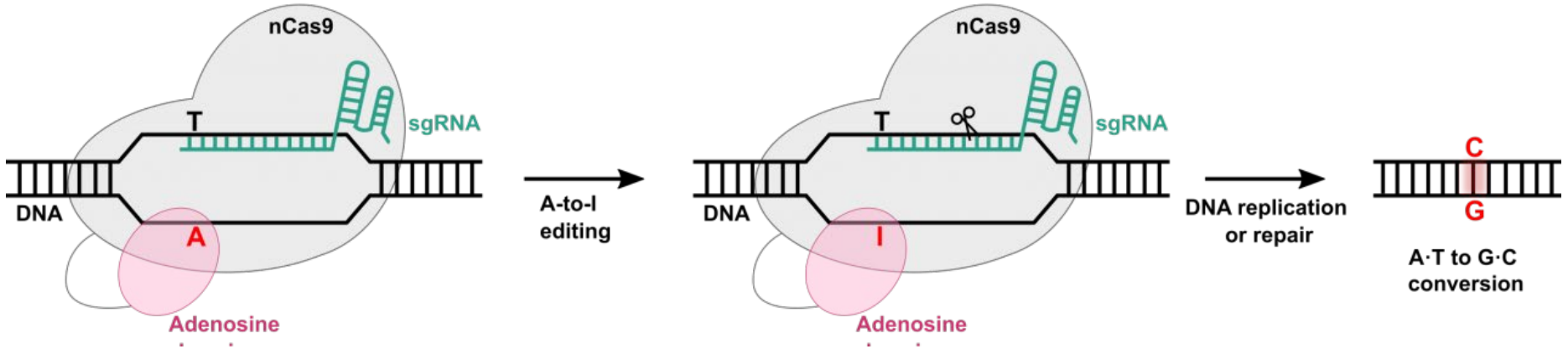
BASE EDITORS

2016



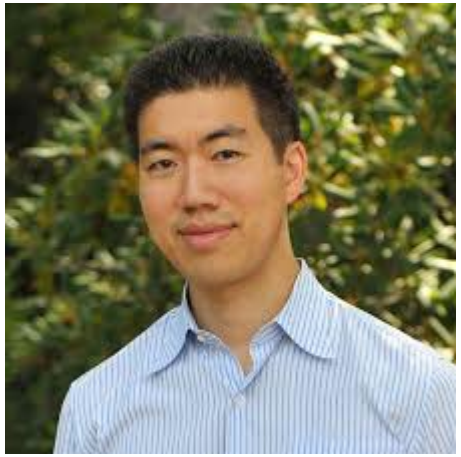
David Liu



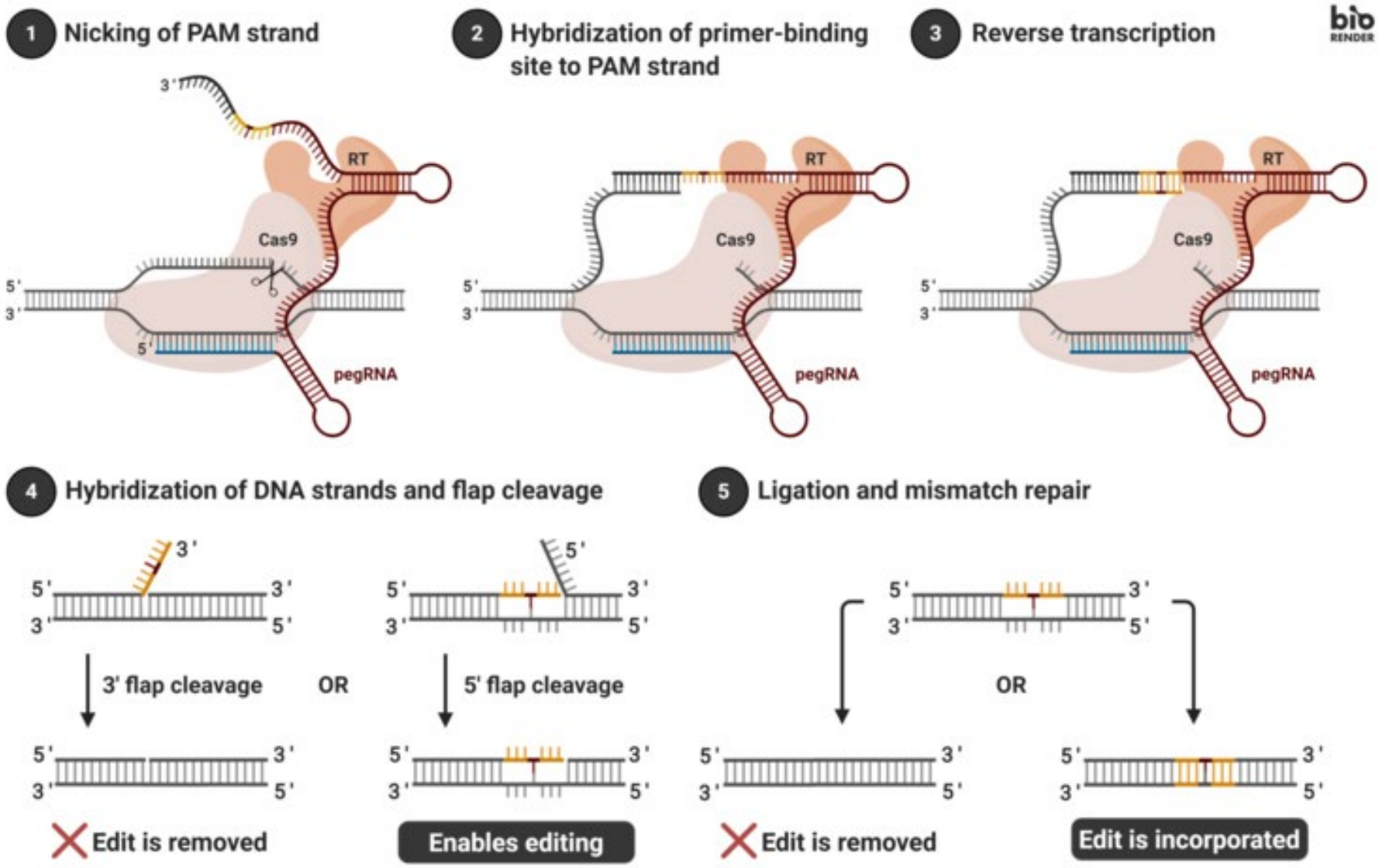


PRIME EDITORS

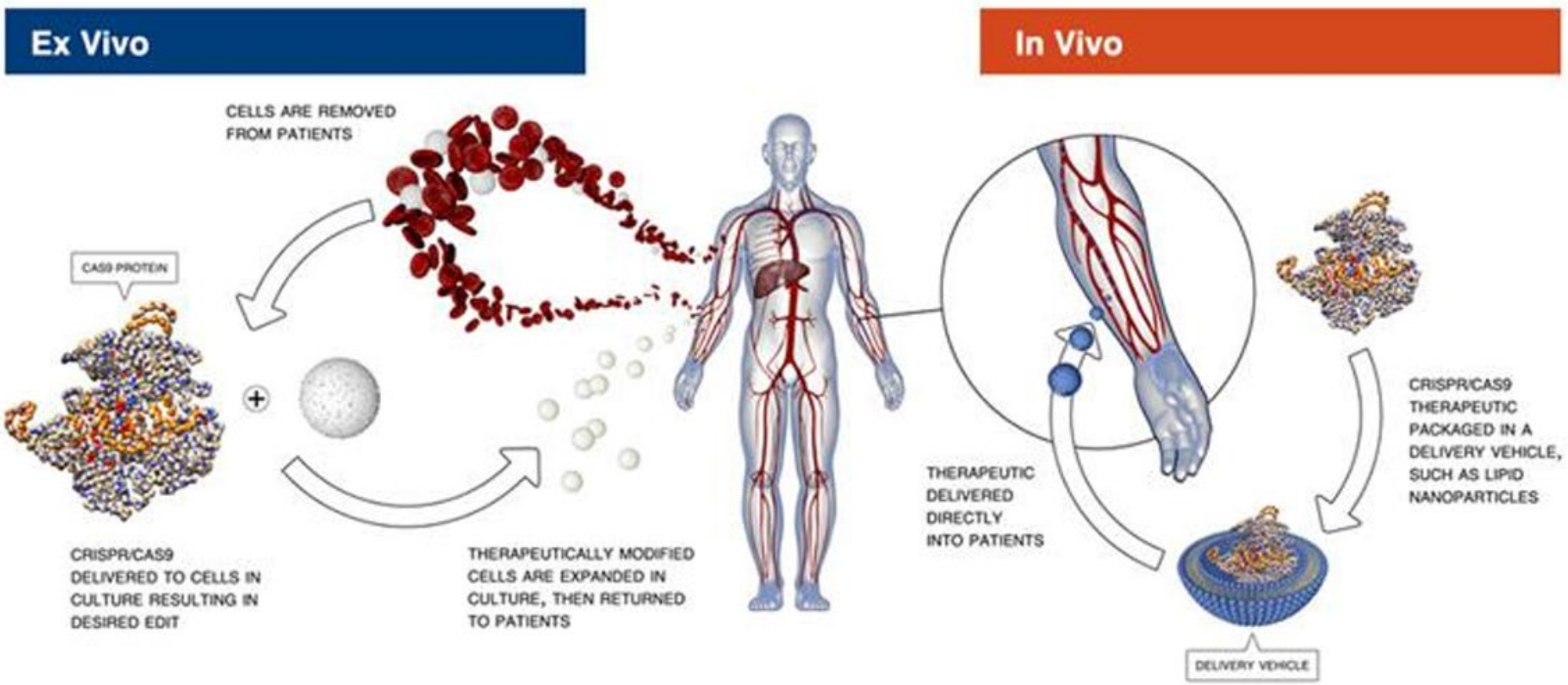
2019



David Liu



CRISPR/CAS9 EN TERAPIA GÉNICA



CRISPR/CAS9 EN TERAPIA GÉNICA

- Del laboratorio a 1ª Terapia Génica aprobada en **11 años**

Trasplante autólogo (*Ex vivo*)

Bacteriofagos Cas3 (*In situ* vejiga)

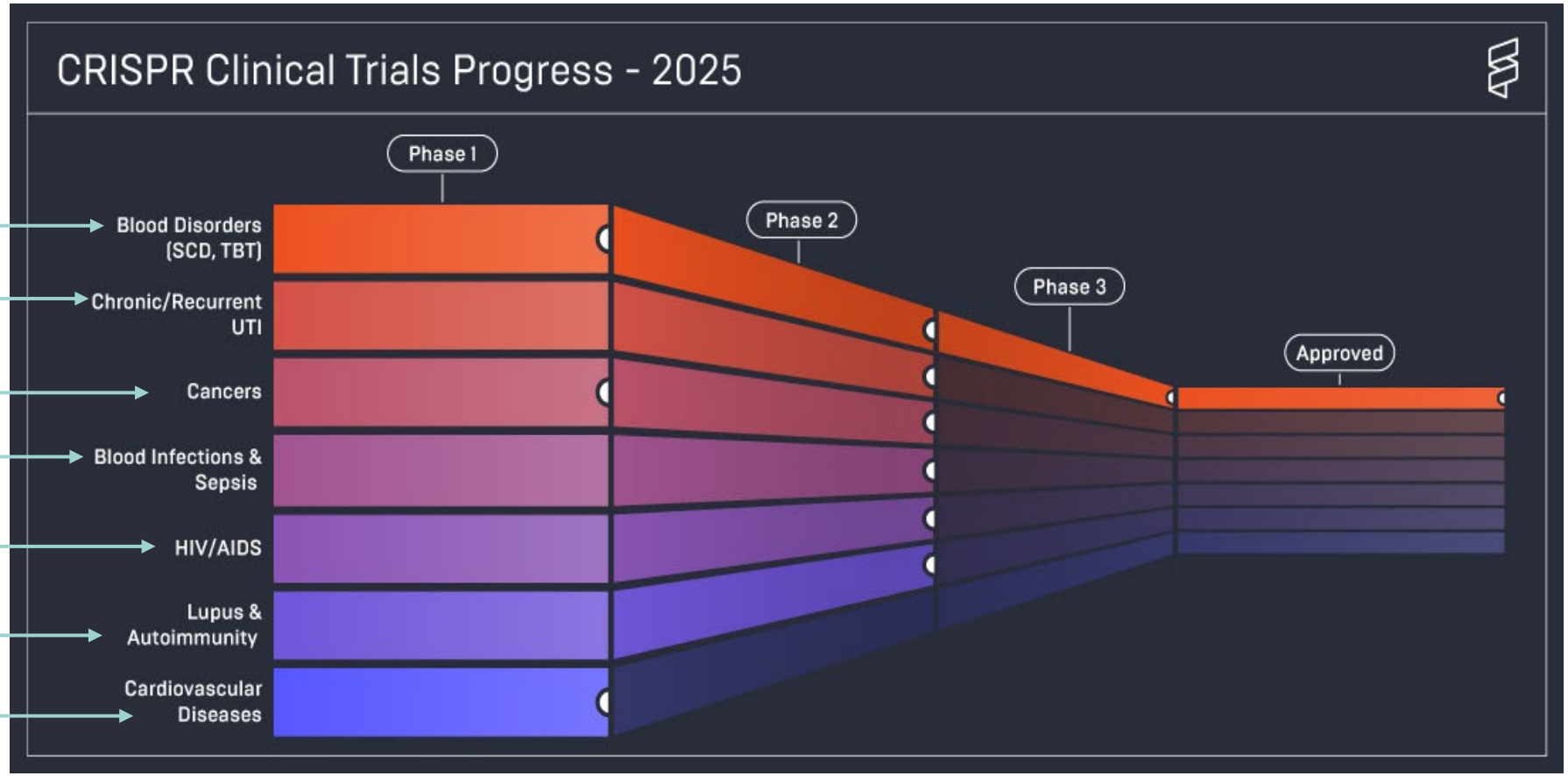
CAR-T autólogos (*Ex vivo*)

Bacteriofagos (*In vivo*)

AAV (*In vivo*)

Alotrasplant cels madre modificadas (*Ex vivo*)

Base-editors. Nanopart. Lip.(*In vivo*)



https://innovativegenomics.org/news/crispr-clinical-trials-2025/

FIN