2021 CRISPR Year in Review

Science Circle
January 29th 2022

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Researcher at Corteva some of whose research is presented here. Not representing the company’s positions.

Nothing should be construed as investment advice or company forward-looking statements

2021 CRISPR Year in Review

Background

Target Genes and Modified Organisms

Human Disease Cure, HIV, bacteria mods, plants

Origin Story

transposon career change

Cautionary Tales

Messing up chromosomes

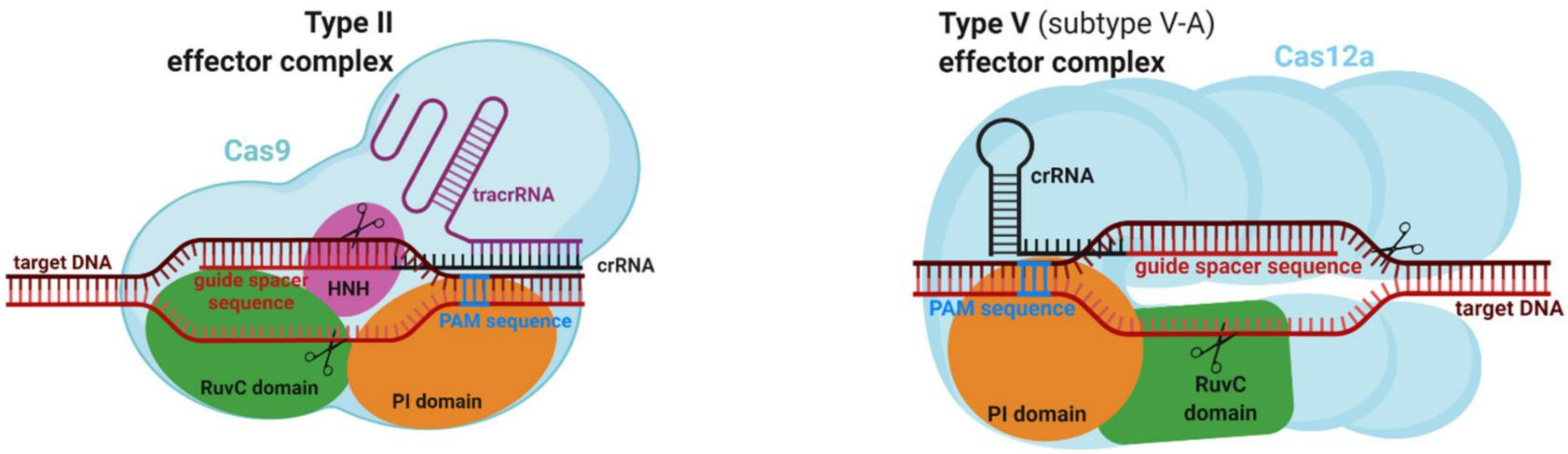
Technology Advances

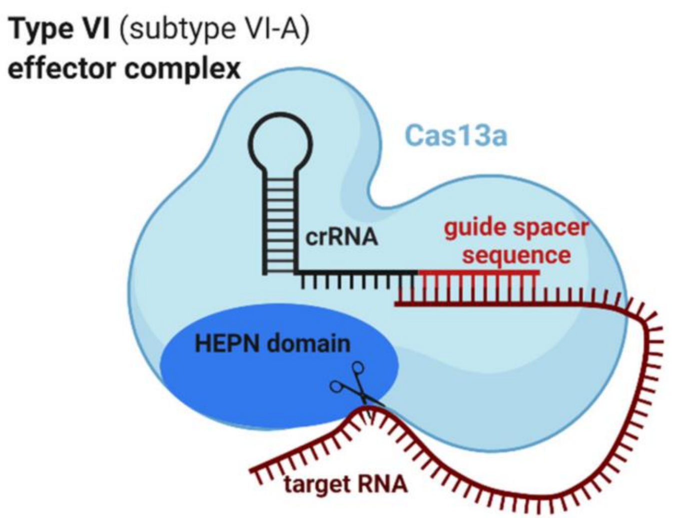
Tissue delivery, Inversions, Plant efficiency, ONYX

New Tools

Payloads, miniCas, COVID screen, reprogramed

2021 CRISPR Year in Review





Cas9 First and most studied and used

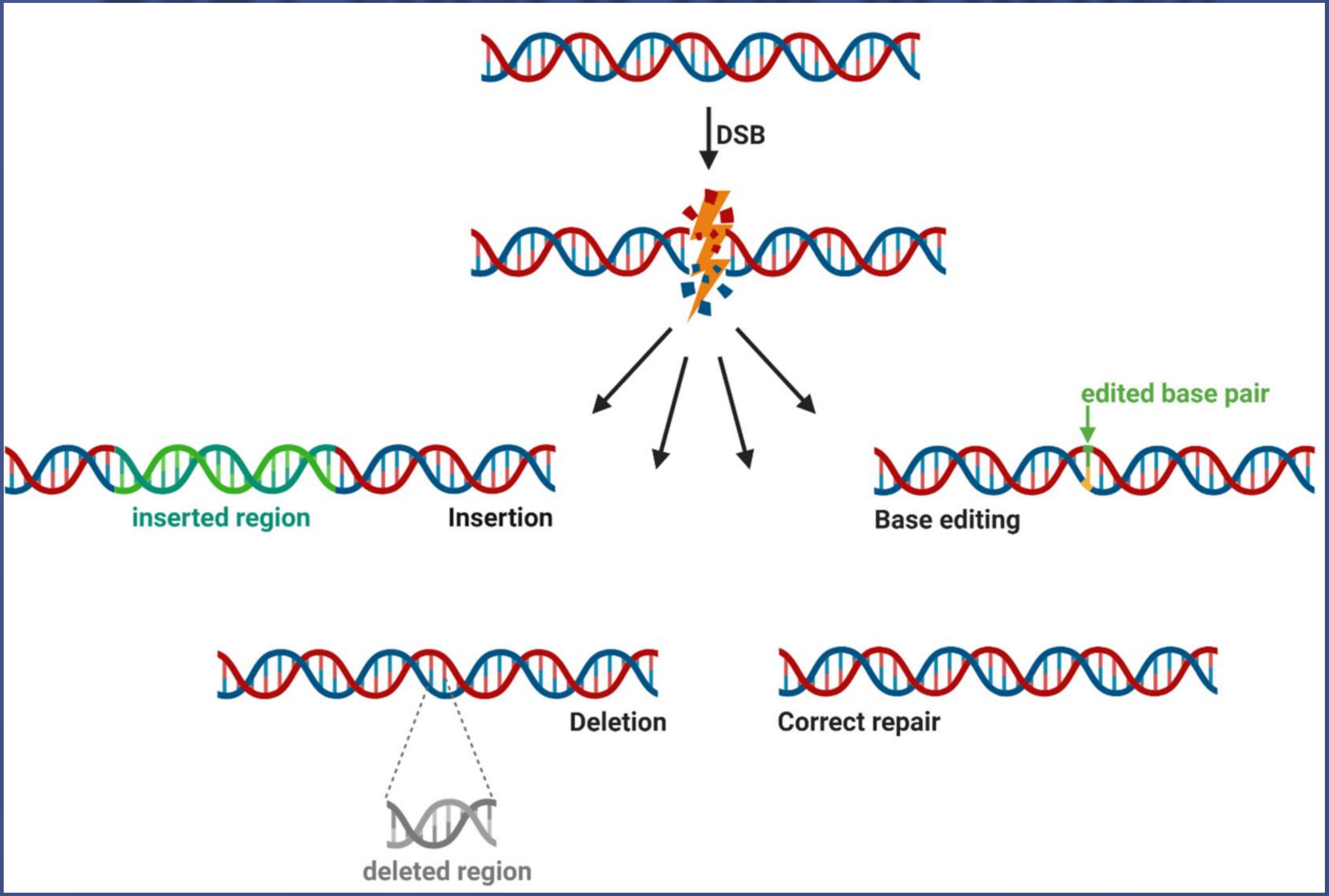
Cas12a Second most well studied (large families of Cas12s)

Cas13a targets RNA

Technique allows us to localize a protein to precise locations via RNA homology

Nidhi, Sweta, et al. "Novel CRISPR–Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives." *International Journal of Molecular Sciences* 22.7 (2021): 3327.

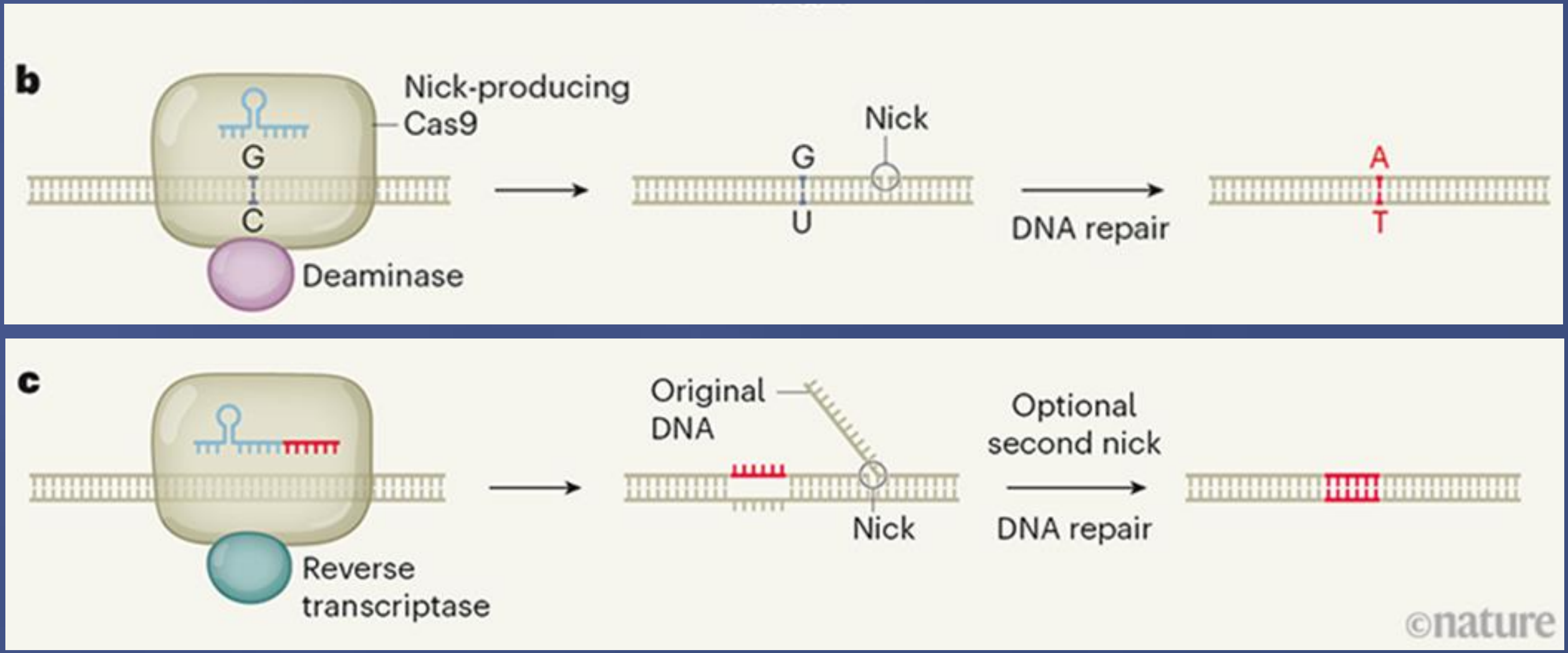
2021 CRISPR Year in Review



A double strand break can modify or add sequences at a target location

Nidhi, Sweta, et al. "Novel CRISPR–Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives." *International Journal of Molecular Sciences* 22.7 (2021): 3327.

2021 CRISPR Year in Review



Base Editing – one nucleotide change

Prime Editing

1) template sequence for the repair
2) small but precise insertions or deletions

No DSBs

Platt, R. J. "CRISPR tool modifies genes precisely by copying RNA into the genome." Nature 576.7785 (2019): 48.

Target Genes and Modified Organisms

"CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis"

Single CRISPR injection to "cure" disease

transthyretin amyloidosis

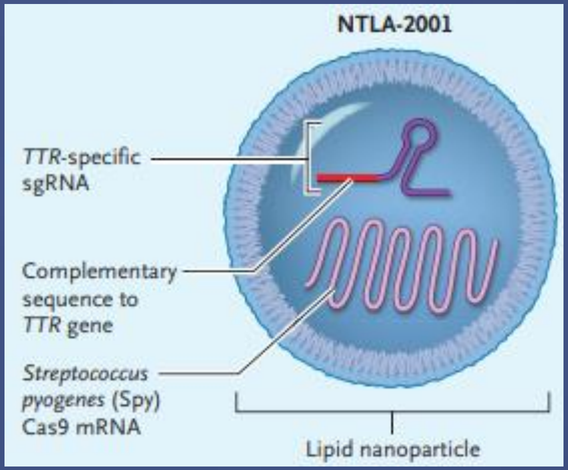
buildup of mutant protein, expressed in liver but circulated in blood

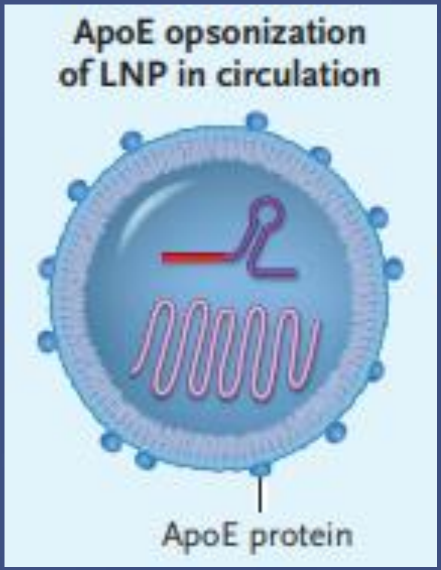
polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, and gastrointestinal features

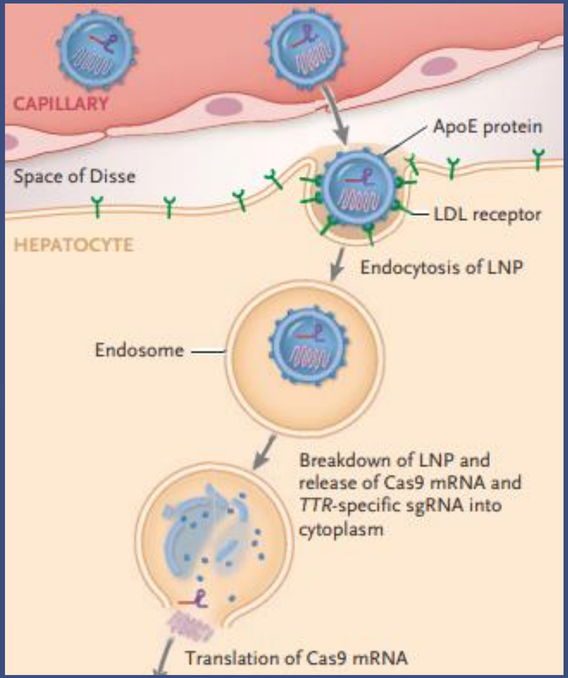
severe diarrhea with malabsorption, cachexia, incapacitating neuropathy, severe cardiac disturbances, and marked orthostatic hypotension

Death usually occurs 5 to 15 years after onset of symptoms

Fig. 1 Delivery







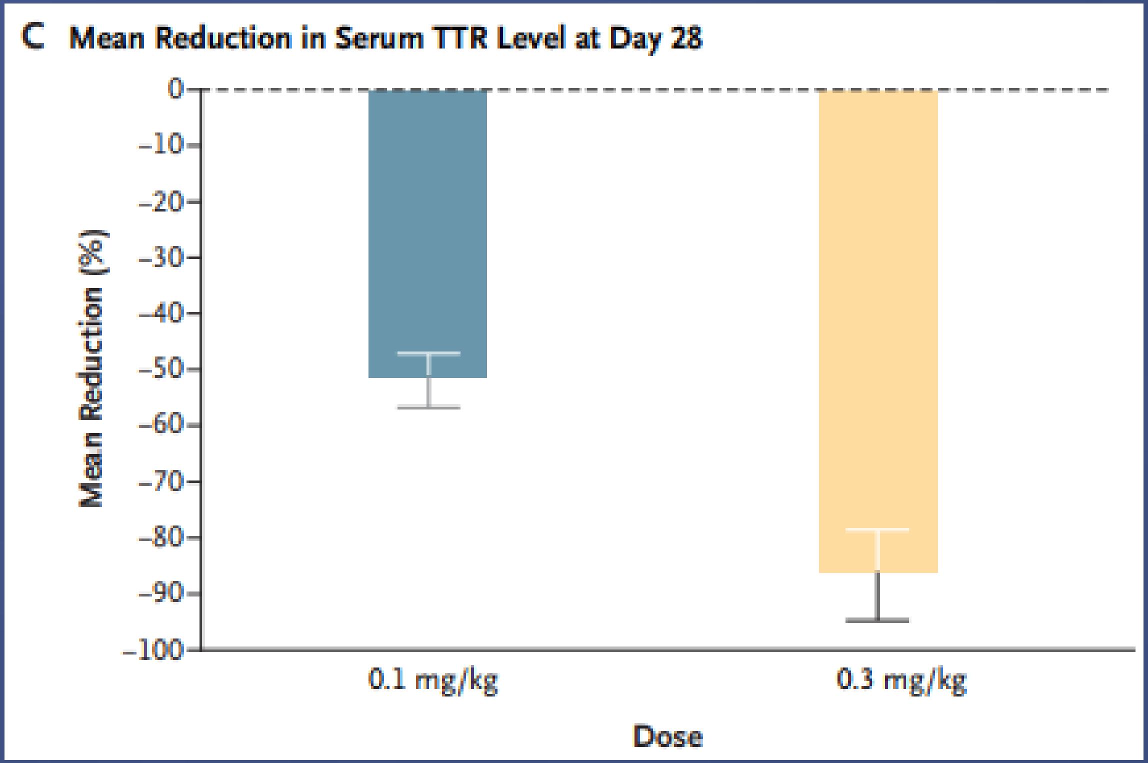
Gillmore, Julian D., et al. *New England Journal of Medicine* 385.6 (2021): 493-502.

Target Genes and Modified Organisms

"CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis"

Single CRISPR injection to "cure" disease

Fig. 2 Reductions from Baseline in Serum TTR Protein Concentration after Infusion of NTLA-2001 in Humans.

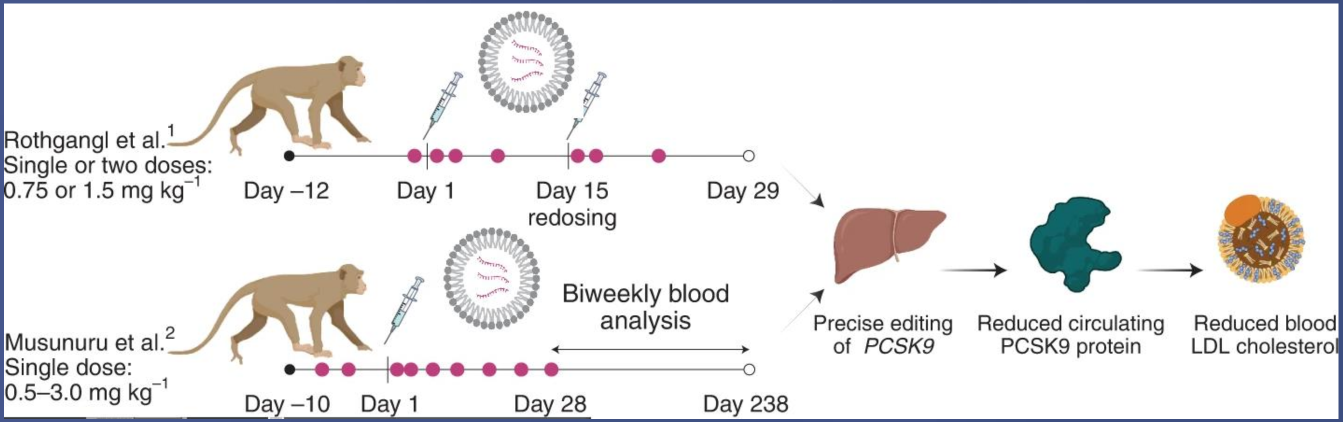


Patrick Doherty volunteered for a new medical intervention of gene-editor infusions for the treatment of genetically-based diseases.

Gillmore, Julian D., et al. *New England Journal of Medicine* 385.6 (2021): 493-502.
June 26, 202111:15 AM ET *All Things Considered* NPR

Target Genes and Modified Organisms

"CRISPR base editing lowers cholesterol in monkeys"

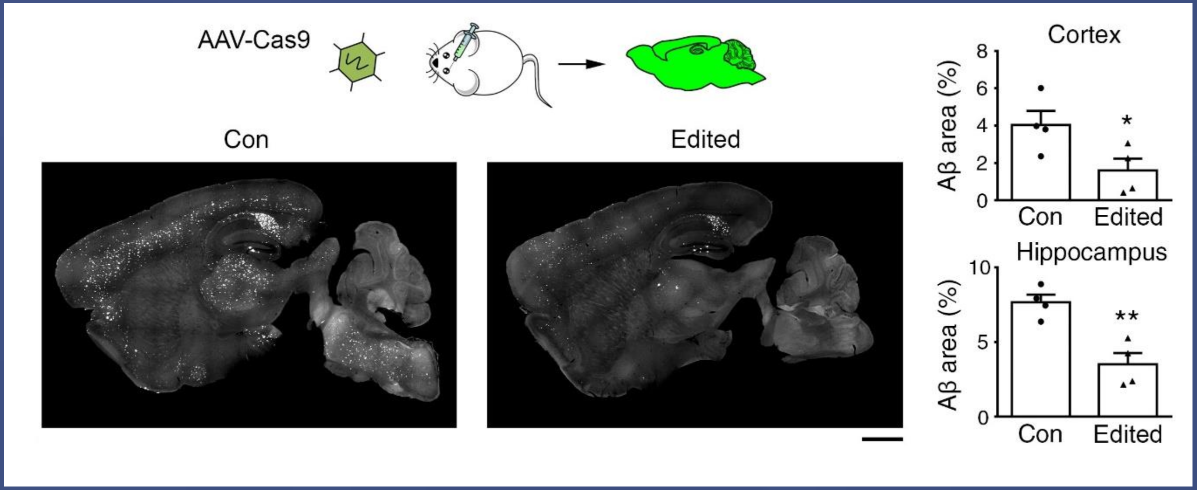


autosomal dominant familial hypercholesterolemia, *PCSK9*

LNP delivery

van Kampen, Sebastiaan Johannes, and Eva van Rooij. *Nature Biotechnology* 39.8 (2021): 920-921 (Review)

"Brain-wide Cas9-mediated cleavage of a gene causing familial Alzheimer's disease alleviates amyloid-related pathologies in mice"



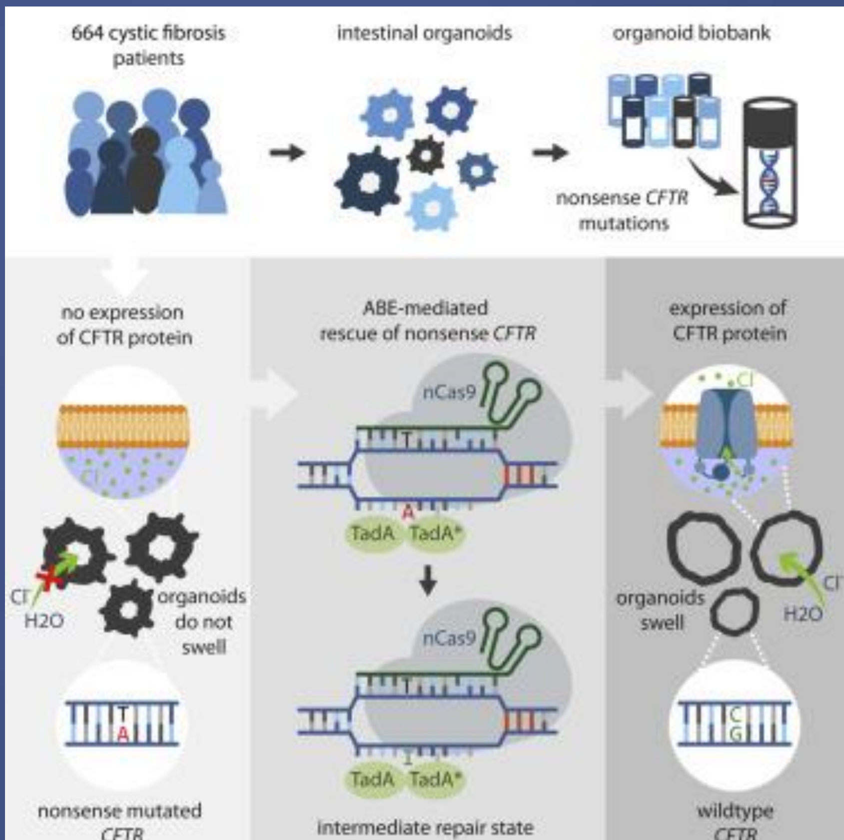
***APP:* AMYLOID BETA A4 PRECURSOR PROTEIN**

Modified adeno-associated virus (AAV) variants that can cross the blood–brain barrier (BBB)

Duan, Yangyang, et al. *Nature Biomedical Engineering* (2021): 1-13.

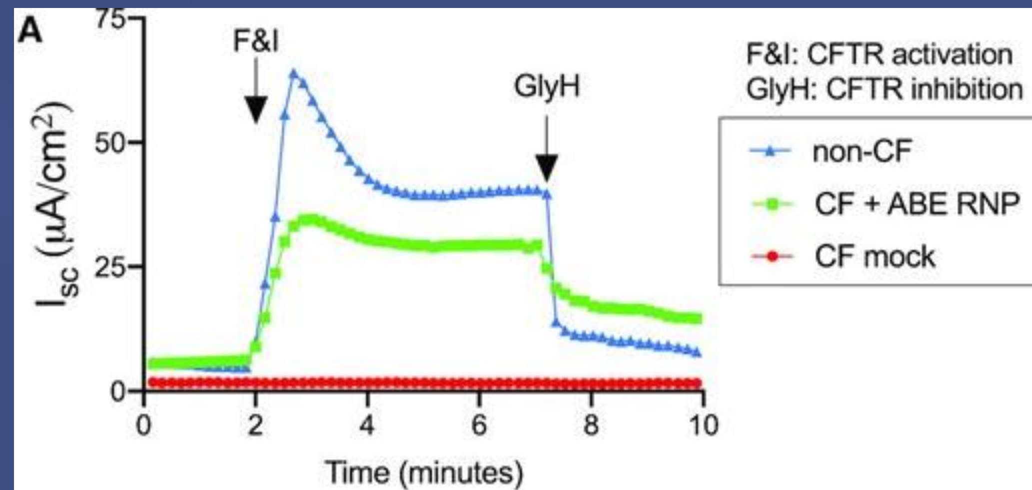
Target Genes and Modified Organisms

"CRISPR-Based Adenine Editors Correct Nonsense Mutations in a Cystic Fibrosis Organoid Biobank" (2020)



Geurts, Maarten H., et al. *Cell Stem Cell* 26.4 (2020): 503-510.

"Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors"



Krishnamurthy, Sateesh, et al. *Nucleic acids research* 49.18 (2021): 10558-10572.

Continuing progress on delivery to lung epithelial cells for therapy

Jiang, Tingting, et al. "Chemical modifications of adenine base editor mRNA and guide RNA expand its application scope." *Nature communications* 11.1 (2020): 1-9.

Target Genes and Modified Organisms

"Transient CRISPR-Cas Treatment Can Prevent Reactivation of HIV-1 Replication in a Latently Infected T-Cell Line"

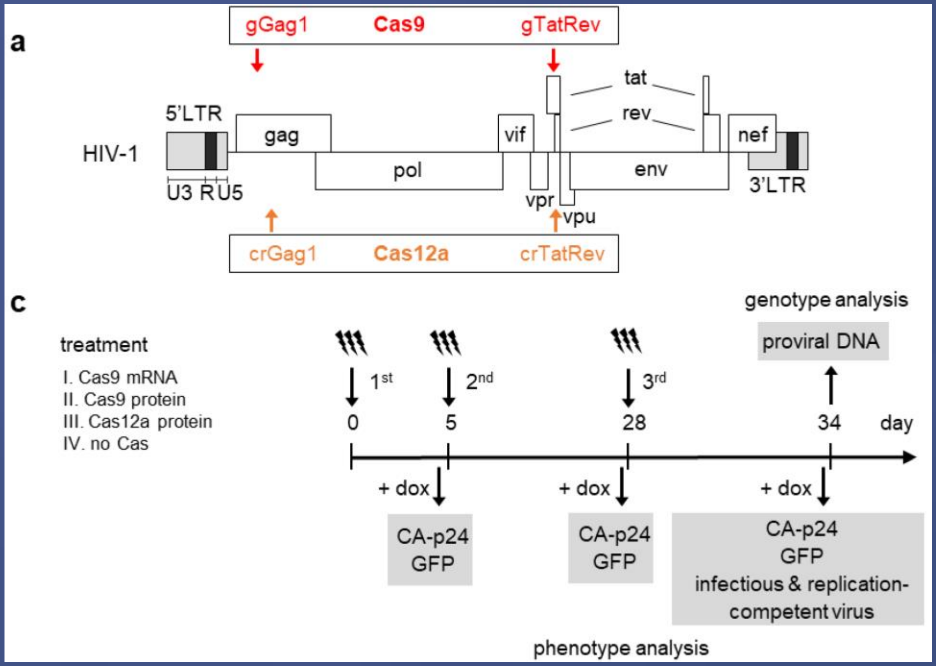


Fig. 1

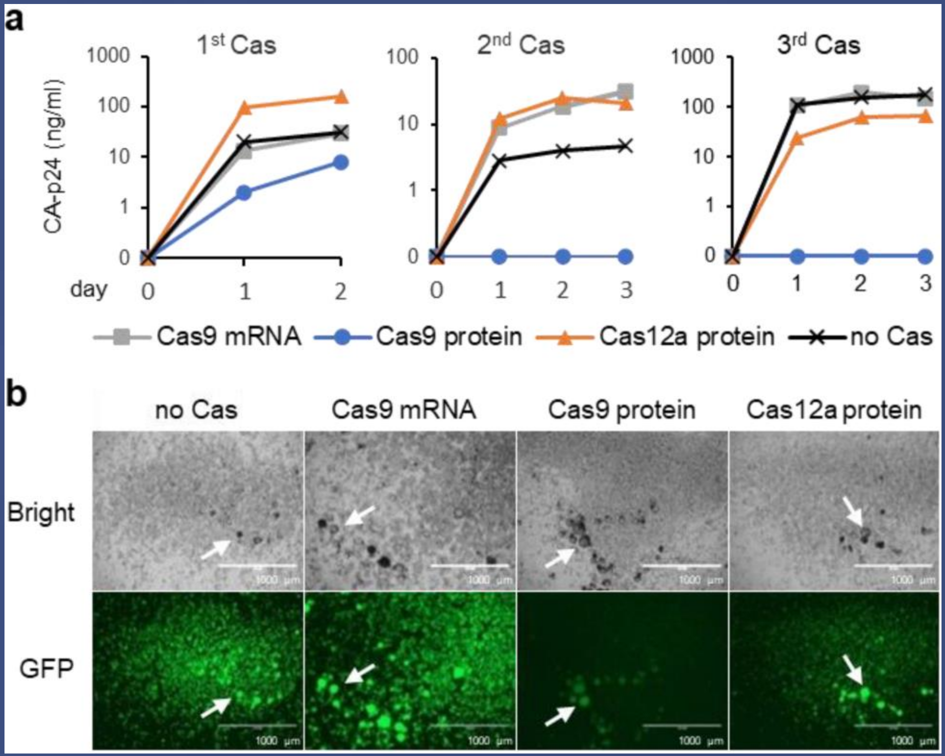


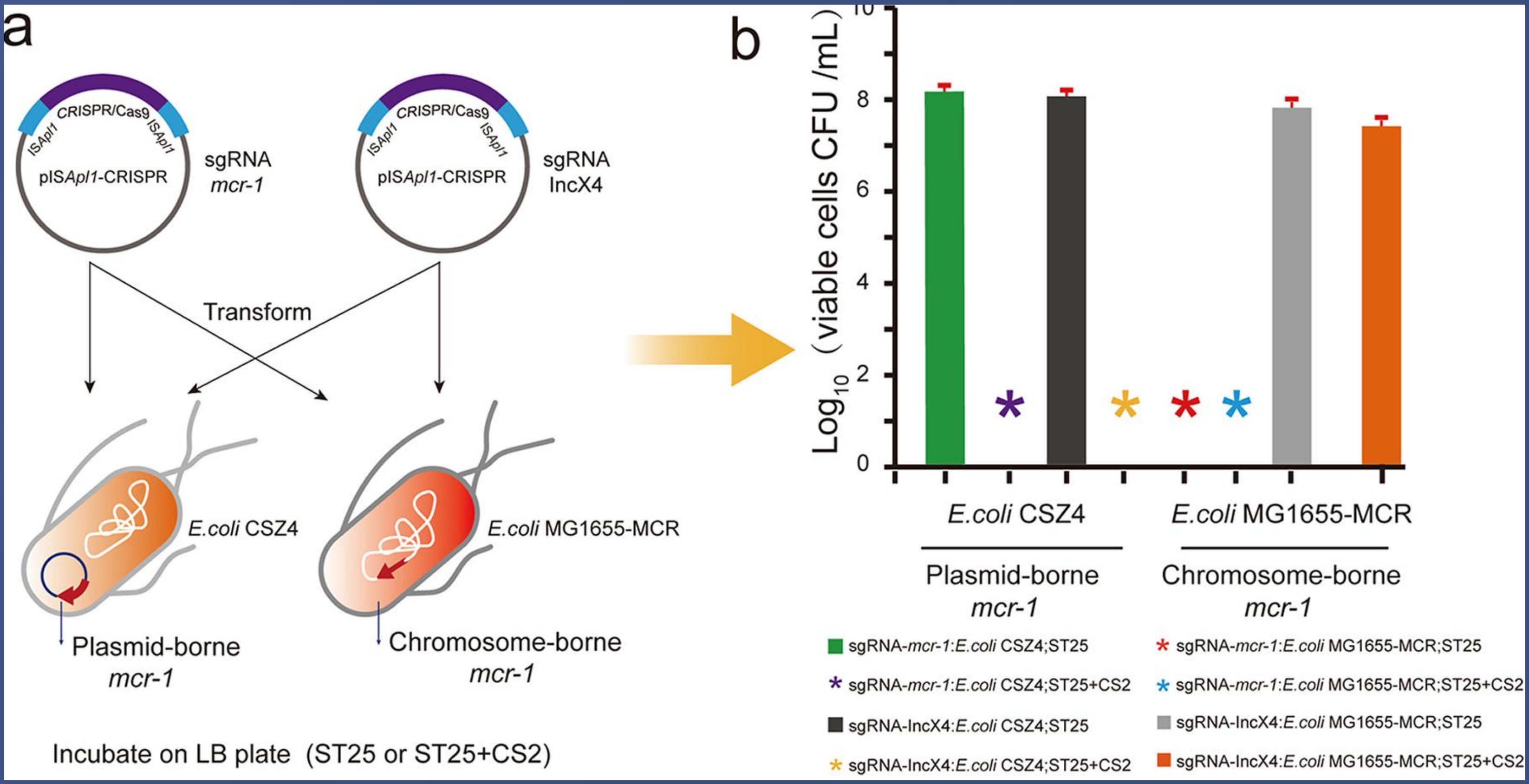
Fig. 2

Multiple short term edits (no introduction of DNA expression constructs or viruses, achieves loss of HIV function

Liu, Ye, et al. Viruses 13.12 (2021): 2461.

Target Genes and Modified Organisms

"A Transposon-Associated CRISPR/Cas9 System Specifically Eliminates both Chromosomal and Plasmid-Borne *mcr-1* in Escherichia coli"



Using transposon delivery systems for CRISPR to target antibiotic resistance, transposons are what frequently spread resistance genes in bacteria populations

He, Yu-Zhang, et al. *Antimicrobial agents and chemotherapy* 65.10 (2021): e01054-21.

Target Genes and Modified Plants

Plant Editing Firsts

Plant	Species	Tech. notes	Gene(s)	Effect
chickpea	*Cicer arietinum*	protoplasts	*4CL7* *RVE7*	Drought Tolerance
rubber tree	*Hevea brasiliensis*	protoplasts	*PDS*	"Albinism"
hop	*Humulus lupulus*	Agro explants	*PDS*	"Albinism"
shēng dì huáng	*Rehmannia glutinosa*	Agro explants	*PDS*	"Albinism"
oil palm	*Elaeis guineensis*	electro-transfection, biolistic particle bombardment	*PDS* *BRI1*	"Albinism" stunting
ergot fungus	*Claviceps purpurea*	Fungal pathogen	*pyr4* and *TrpE*	Decreased virulence

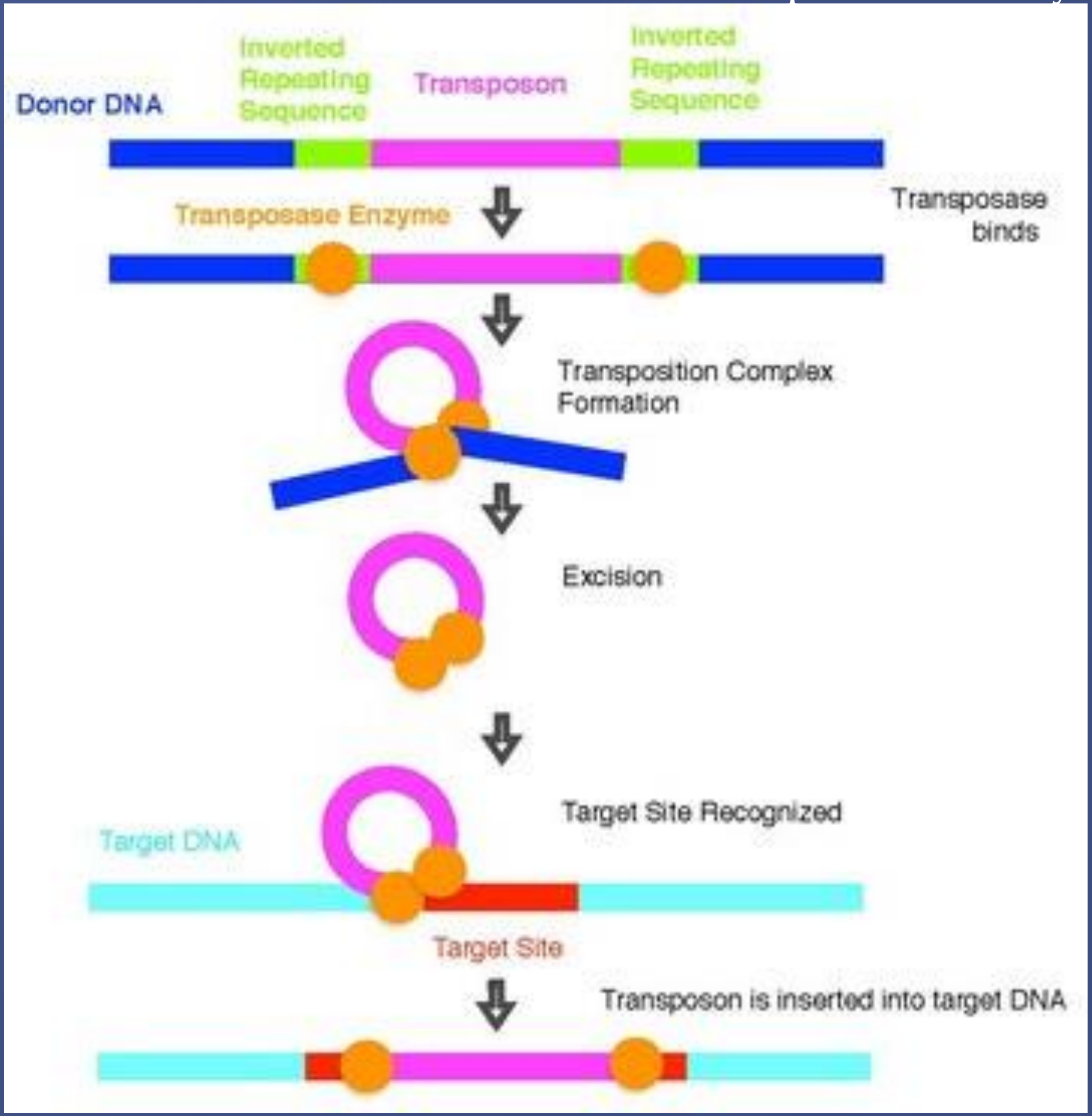
Target Genes and Modified Plants

Notable Plant Edits

Plant	Gene	Effect	Value
cherry tomato	*eIF4E2* in a	resistance to pepper veinal mottle virus	increased yield
soy	NBS-LRR	Novel resistances	increased yield
tomato	*MAX1*	Resistance to parasitic root weed	increased yield
banana	*DMR6*	Resistance to banana Xanthomonas wilt	increased yield
potato	*DND1,CHL1, DMR6*	Resistance to late blight	increased yield
rapeseed	*SHP1* and *SHP2*	Decreased shattering of seed pods during mechanical harvest	increased yield
grapefruit	*LOB1*	Resistance to canker	enhanced production
banana	*ACO1*	reduction of endogenous ethylene production	enhanced shelf life
grapevine	*PAT1 (more)*	Enhanced cold tolerance	env. stability
Rice	*RLCK241 (more)*	Enhanced salt tolerance	env. stability

Origin Story

Transposon Life Cycle



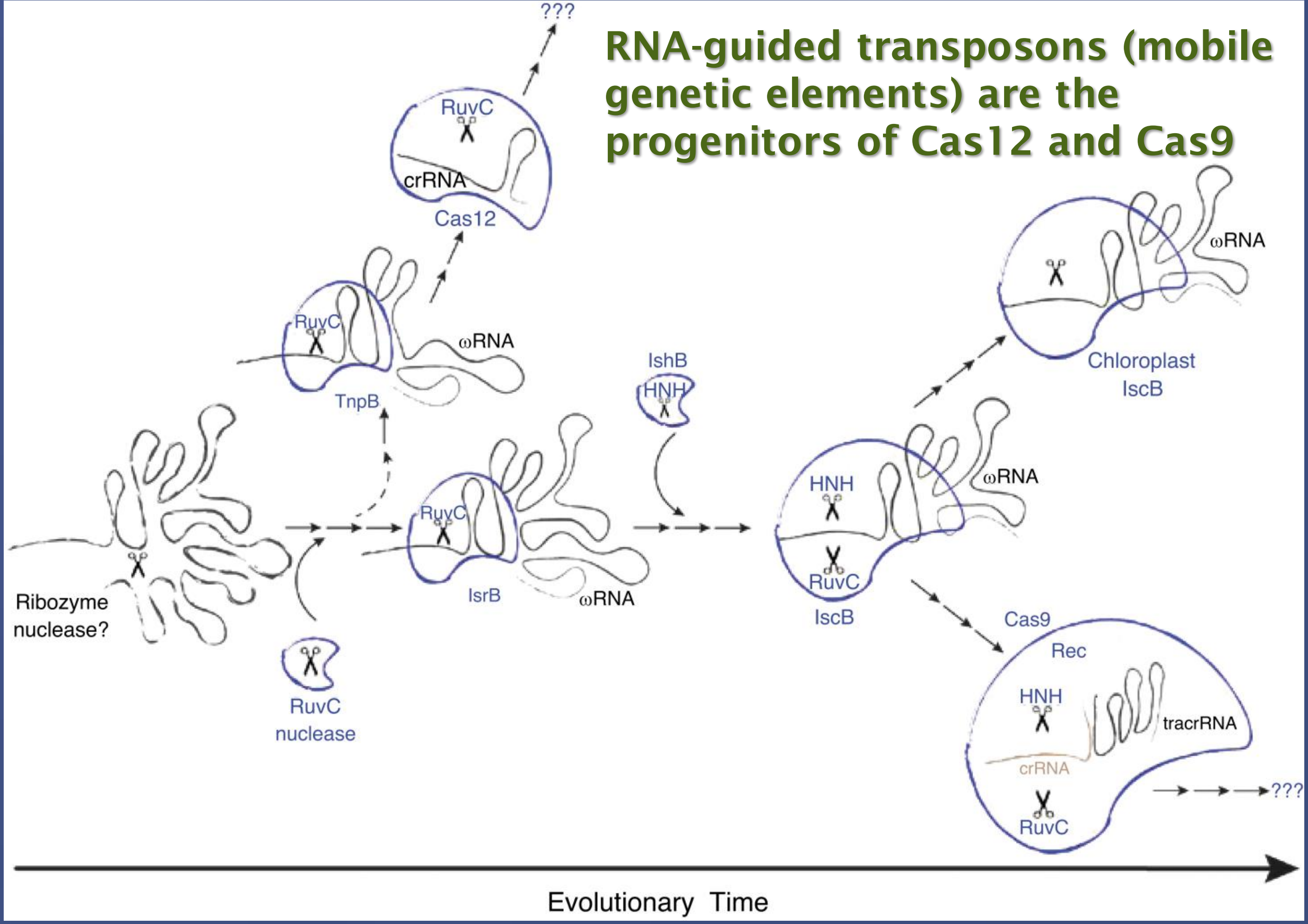
Transposase cuts element out and pastes into new location (no expansion in copy number)

Second gene in bacterial elements is dispensable and unknown function

https://en.wikipedia.org/wiki/DNA_transposon

Origin Story

"The widespread IS200/IS605 transposon family encodes diverse programmable RNA-guided endonucleases"

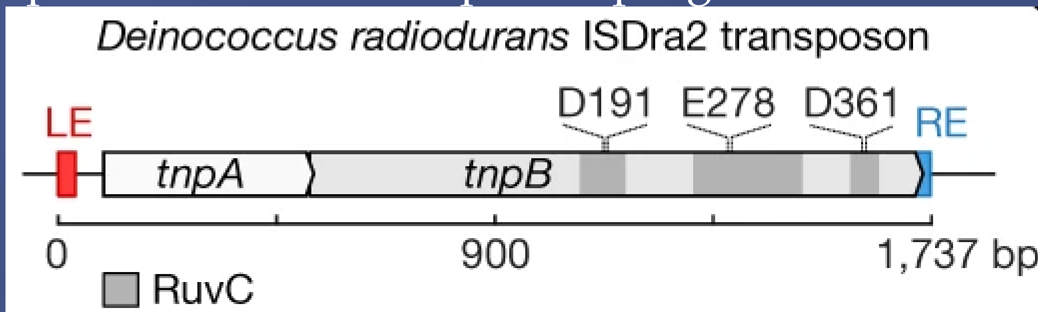


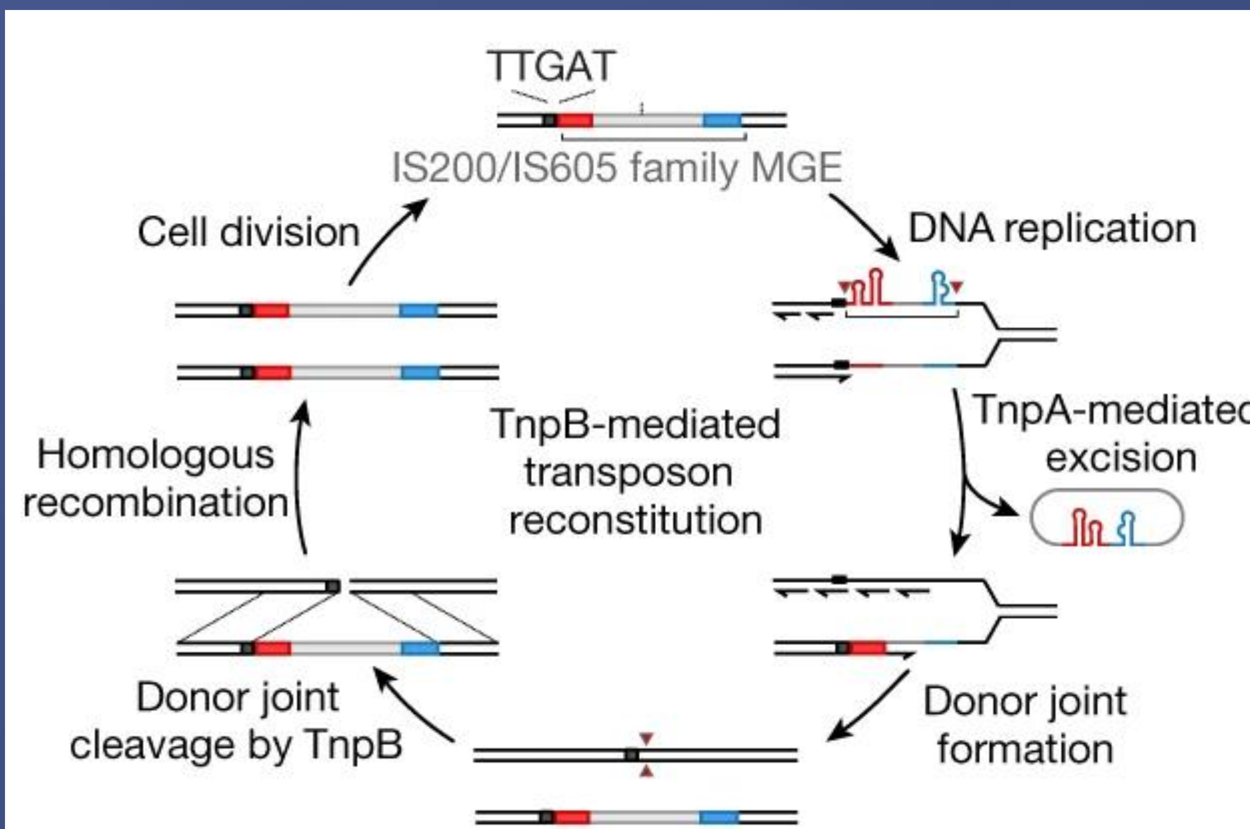
https://www.liebertpub.com/doi/full/10.1089/crispr.2021.29137.knl

Altae-Tran, Han, et al. *Science* 374.6563 (2021): 57-65.

Origin Story

"Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease"





By recleaving its "sister-site" and promoting recombination, it has a "backup" in case the excised copy fails.

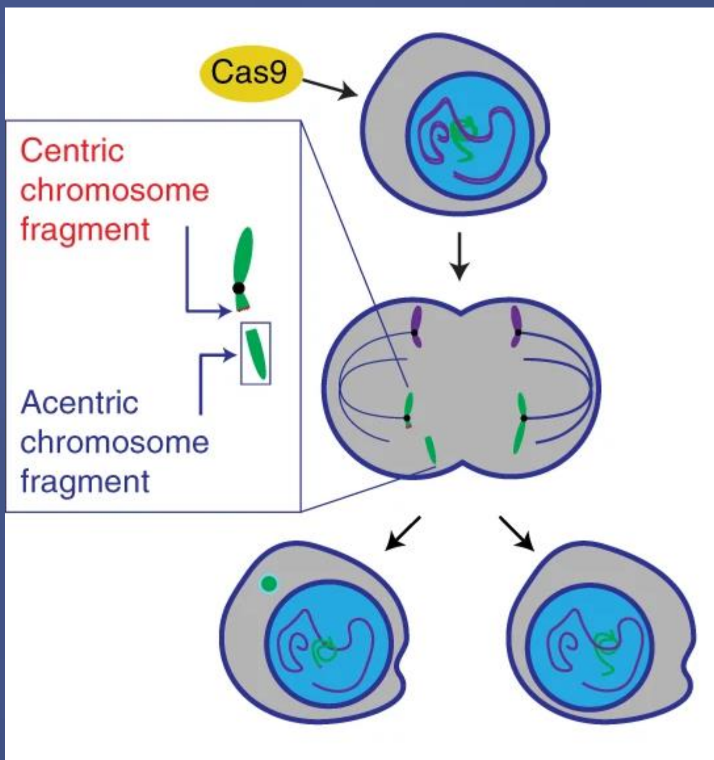
Also, explains how the copy number of the "non-replicative" transposon actually increases

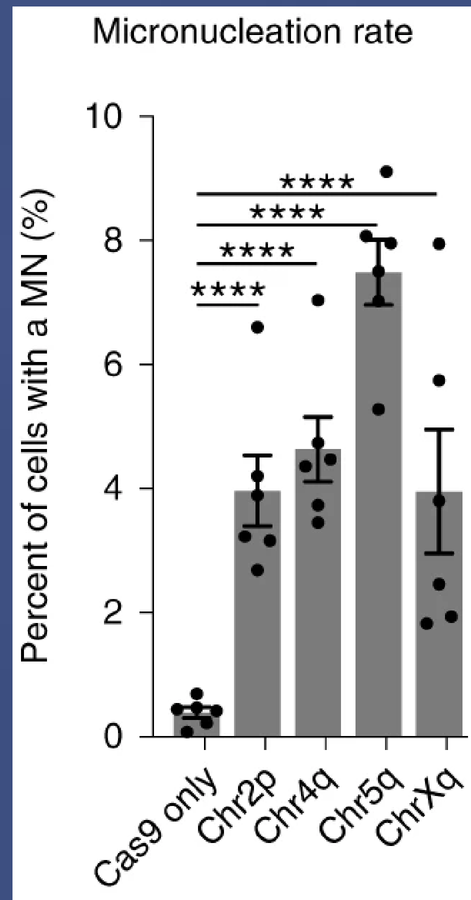
2 copies to 3 copies

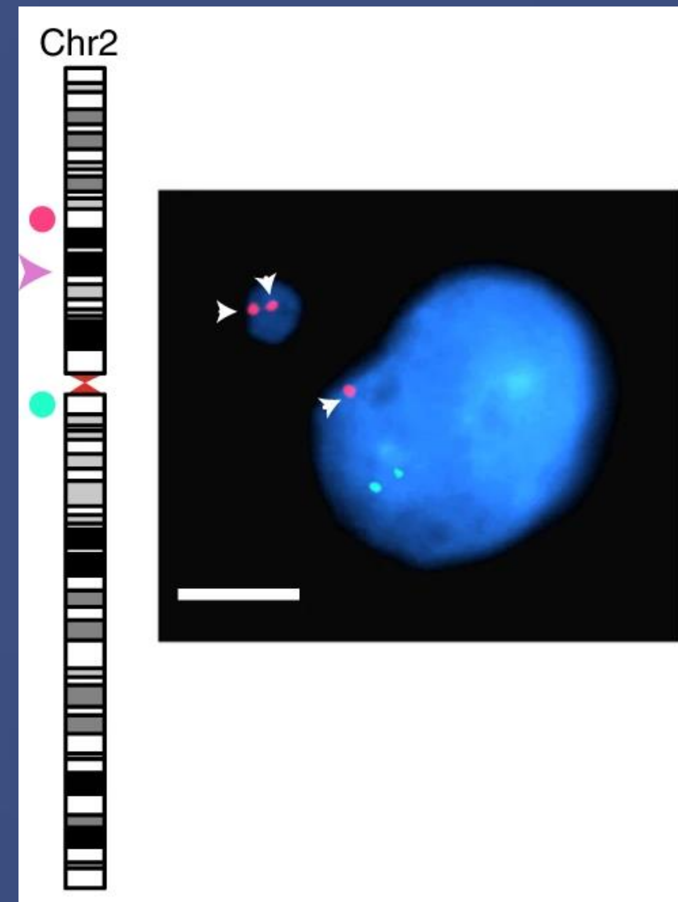
Karvelis, Tautvydas, et al. *Nature* 599.7886 (2021): 692-696.

"Cas9 did what?!?"

"Chromothripsis as an on-target consequence of CRISPR–Cas9 genome editing"





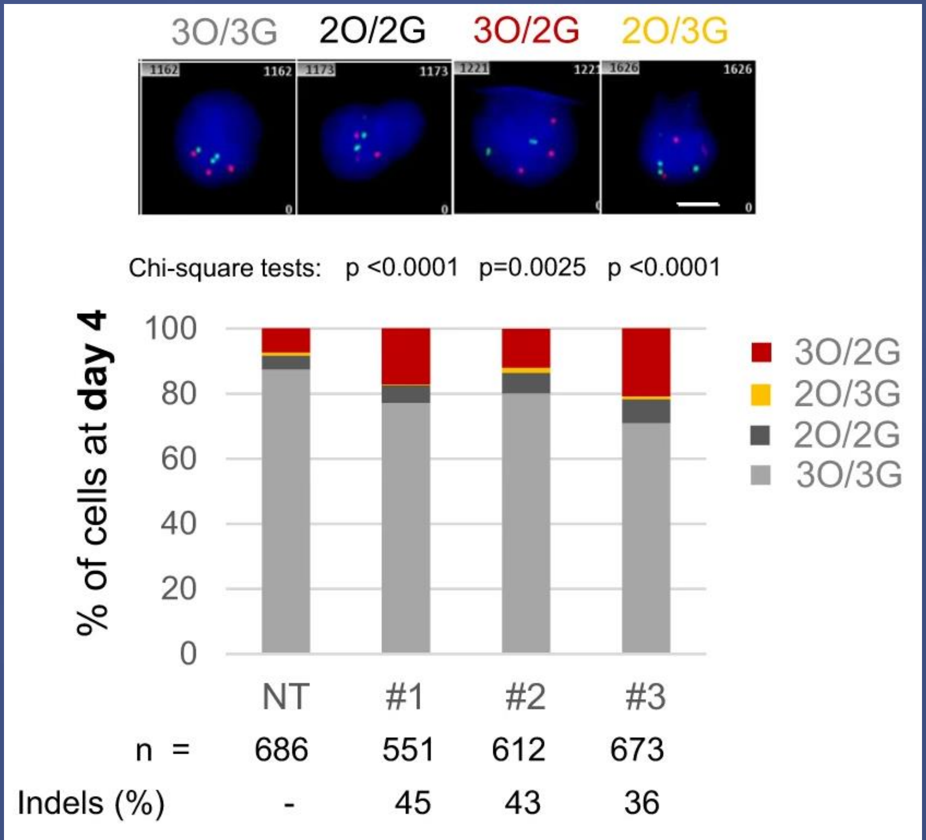


The Cas9 DSB can lead to a cascade of consequences for genome integrity which PERSIST in multiple generations of cells. These are hallmarks of cancer cells.

Leibowitz, Mitchell L., et al. *Nature Genetics* 53.6 (2021): 895-905.

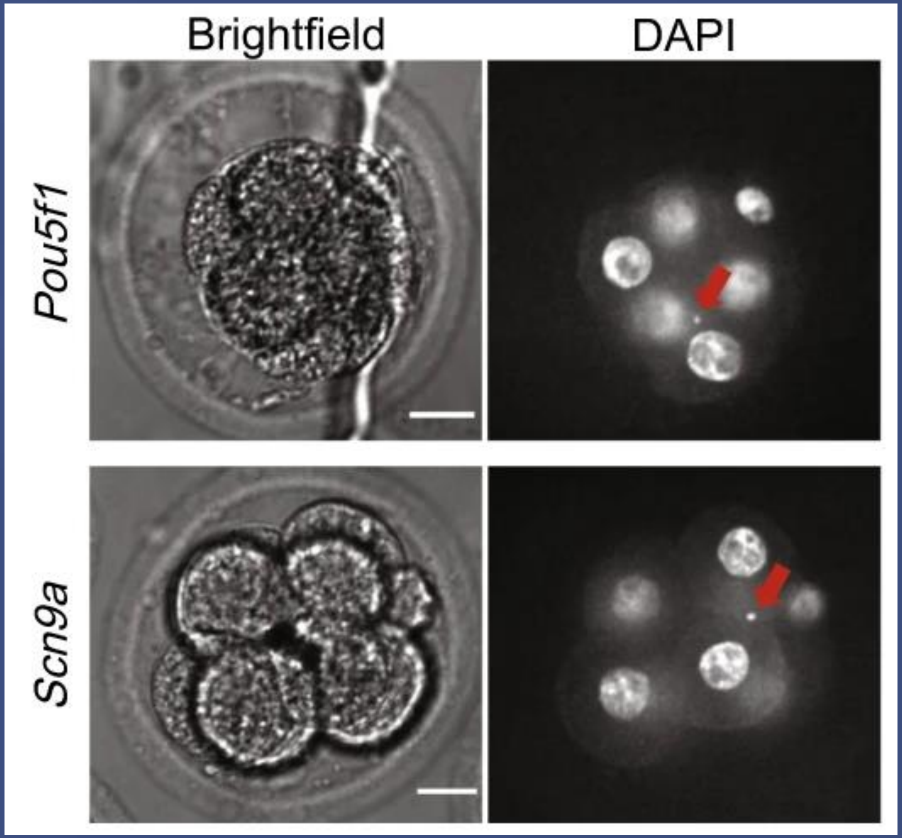
"Cas9 did what?!?"

"CRISPR-Cas9 globin editing can induce megabase-scale copy-neutral losses of heterozygosity in hematopoietic cells"



Boutin, Julian, et al. *Nature Communications* 12.1 (2021): 1-12. (human immortal cell line)

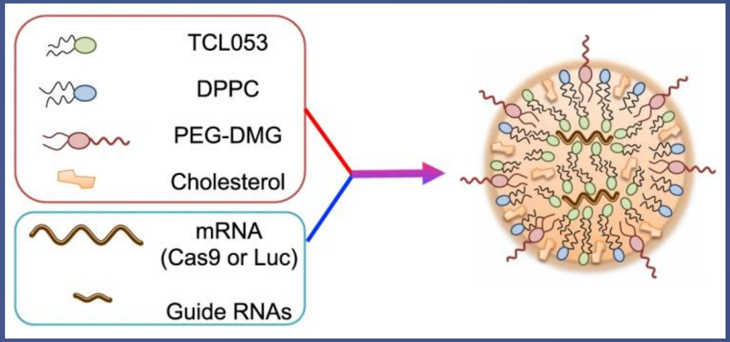
"Whole chromosome loss and genomic instability in mouse embryos after CRISPR-Cas9 genome editing"

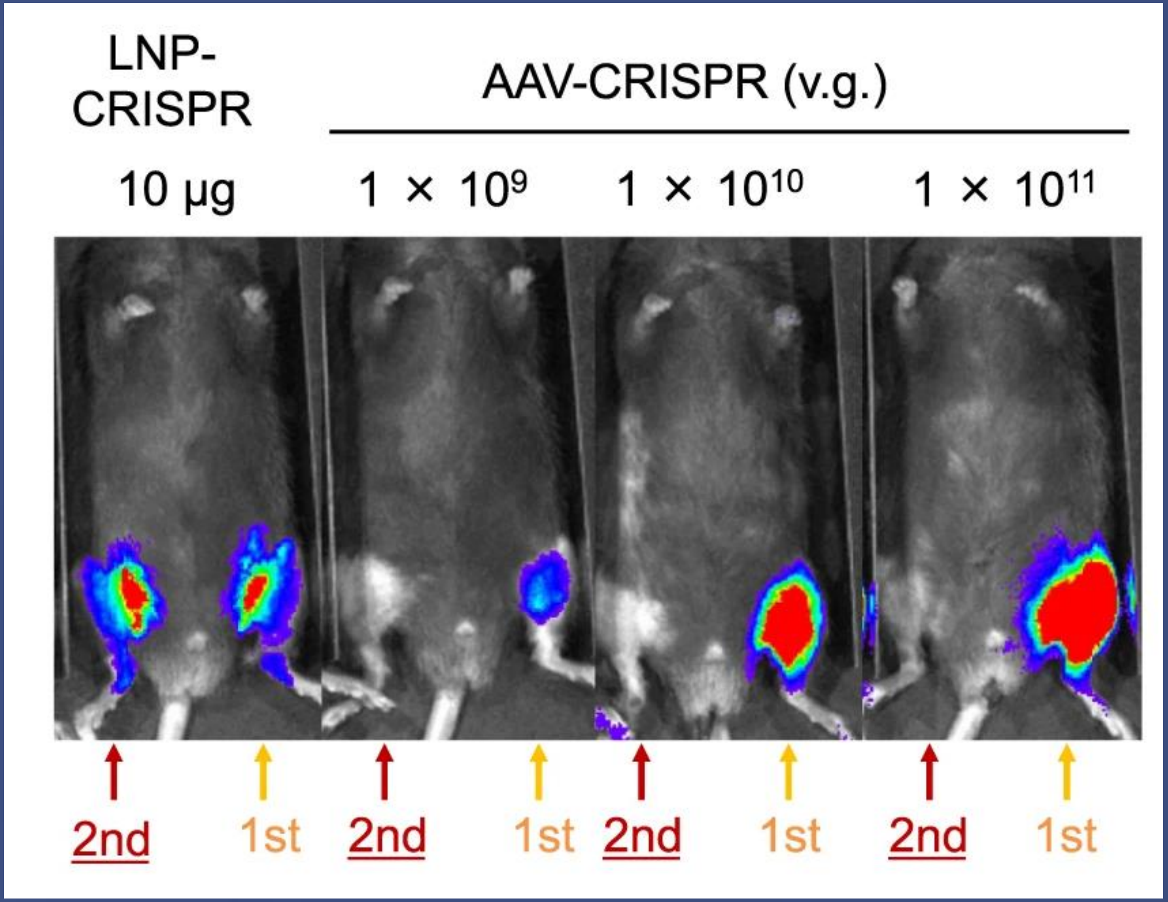


Papathanasiou, Stamatis, et al. *Nature communications* 12.1 (2021): 1-7. (mouse embryos)

Technology Advances

"Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice"



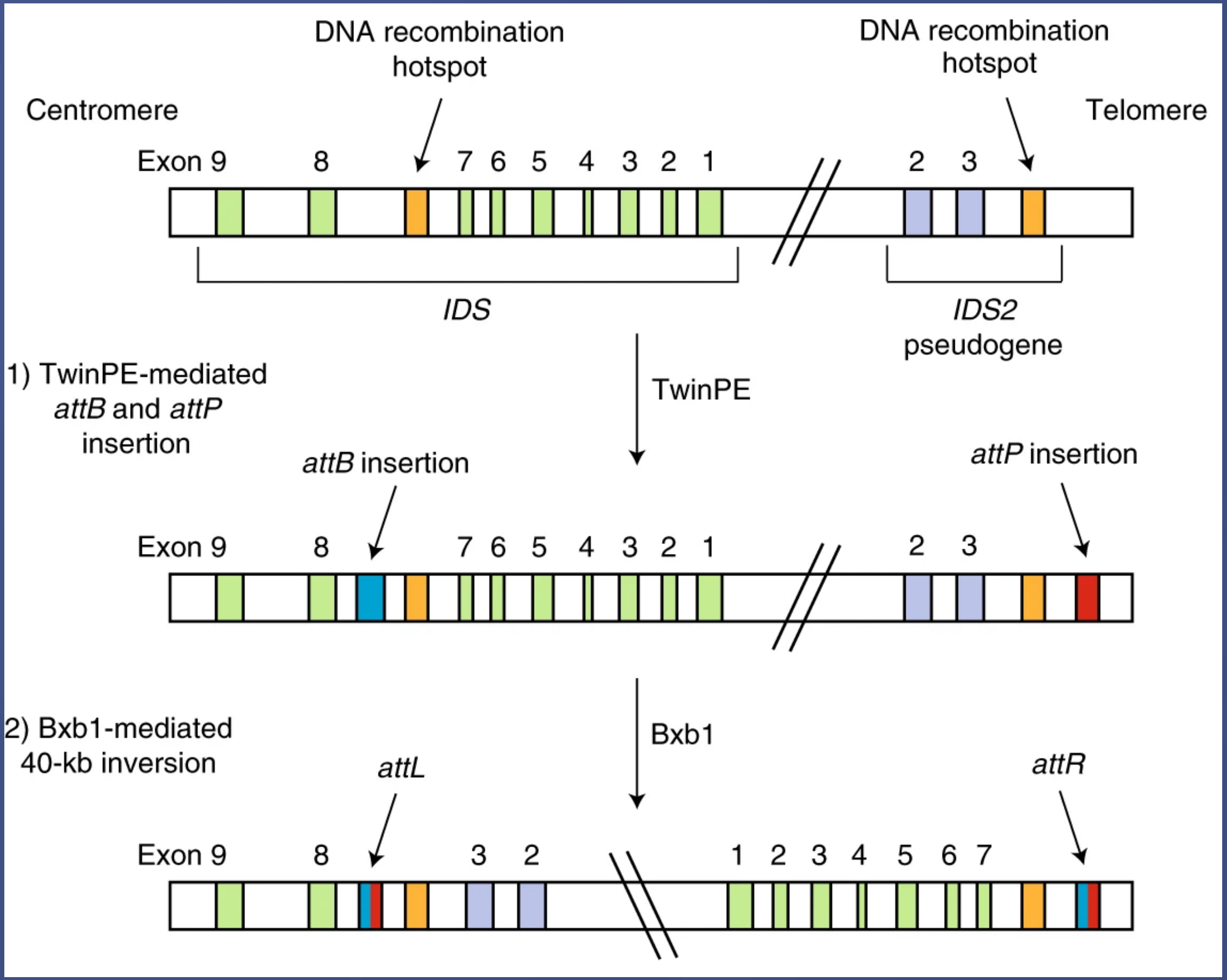


Low immunogenicity means more injections of same gene editing but ALSO to treat multiple diseases

Kenjo, Eriya, et al. ***Nature communications*** **12.1 (2021): 1-13.**

Technology Advances

"Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing"

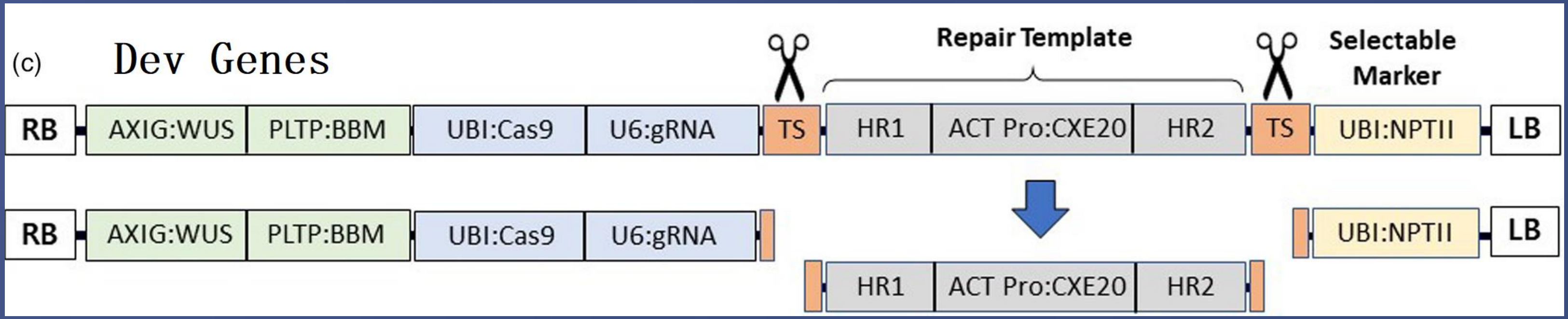


Allows for correction of a uniquely problematic and difficult class of genetic mutations

Anzalone, Andrew V., et al. *Nature biotechnology* (2021): 1-10.

Technology Advances

"Advances in Agrobacterium transformation and vector design result in high-frequency targeted gene insertion in maize"

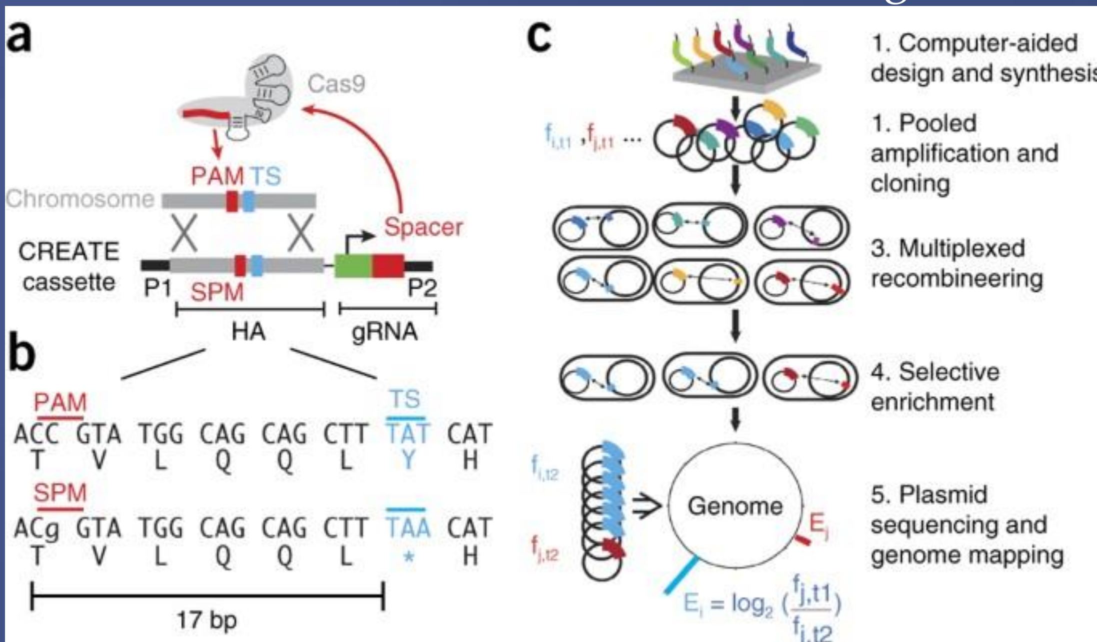


Peterson, Dave, et al. *Plant Biotechnology Journal* (2021).

Since 2016, 100-fold increase in quality transgenic plant development

Technology Advances

"Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering"



Massive parallel edits in a population to screen for changes. Find improved bacteria and yeast for X.

A push-button, benchtop biofoundry.

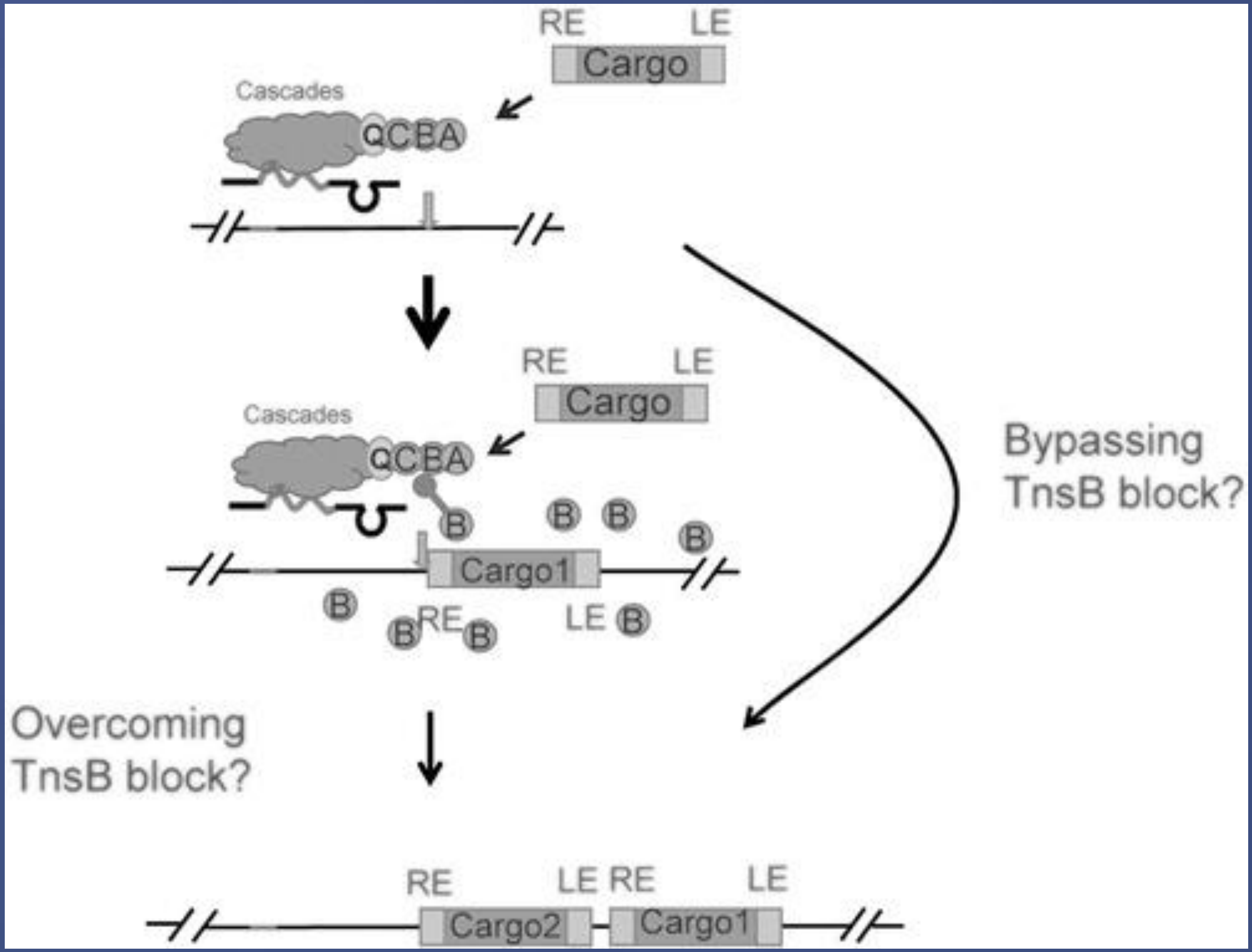
The Onyx Digital Genome Engineering Instrument automates all aspects of large-scale, massively parallel genome engineering experiments — including the cell transformation, CRISPR-based genome engineering, cell growth, and cell recovery — all at your benchtop, all push-button easy.

https://www.inscripta.com/products/onyx-instrument

Garst, Andrew D., et al. *Nature biotechnology* 35.1 (2017): 48-55.

New Tools

"CRISPR-Associated Transposase System Can Insert Multiple Copies of Donor DNA into the Same Target Locus"



Single Delivery but with multiple payloads delivered into one site.

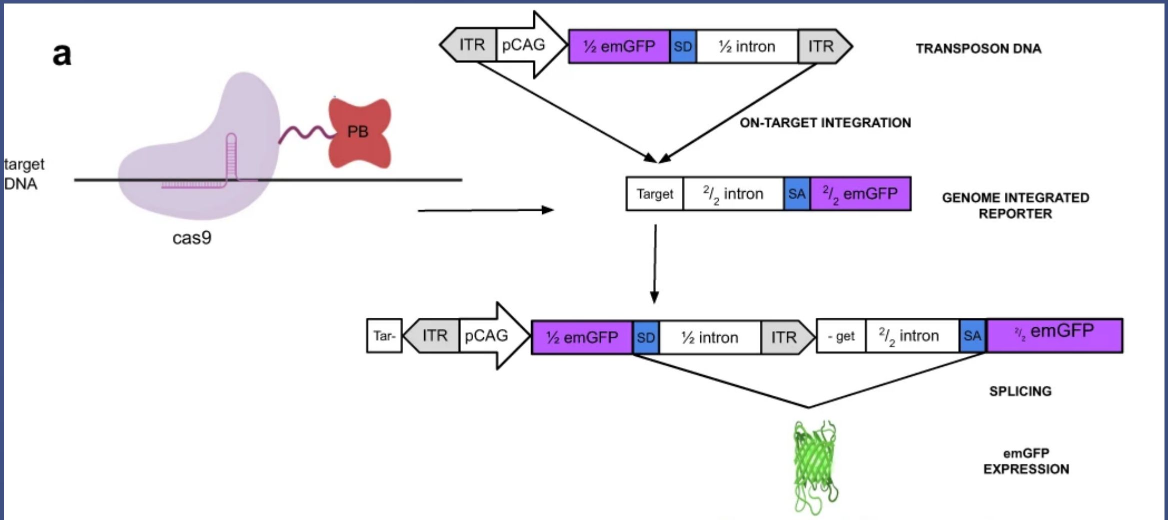
(only in bacteria so far)

Yang, Junjie, et al. *The CRISPR Journal* 4.6 (2021): 789-798..

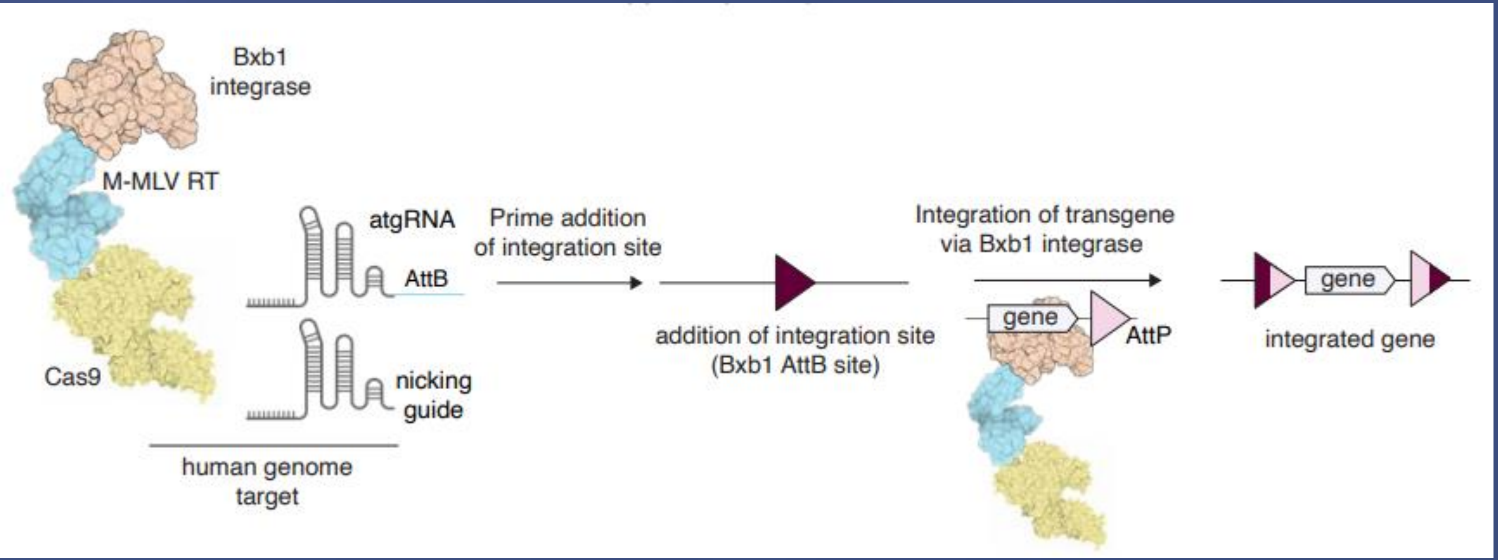
New Tools

“Find and cut-and-transfer (FiCAT) mammalian genome engineering”

Pallarès-Masmitjà, Maria, et al. *Nature communications* 12.1 (2021): 1-9.



“Drag-and-drop genome insertion without DNA cleavage with CRISPR-directed integrases”



Directed payload insertions (>30kb) and no double-strand breaks

Ioannidi, Eleonora I., et al. *bioRxiv* (2021).

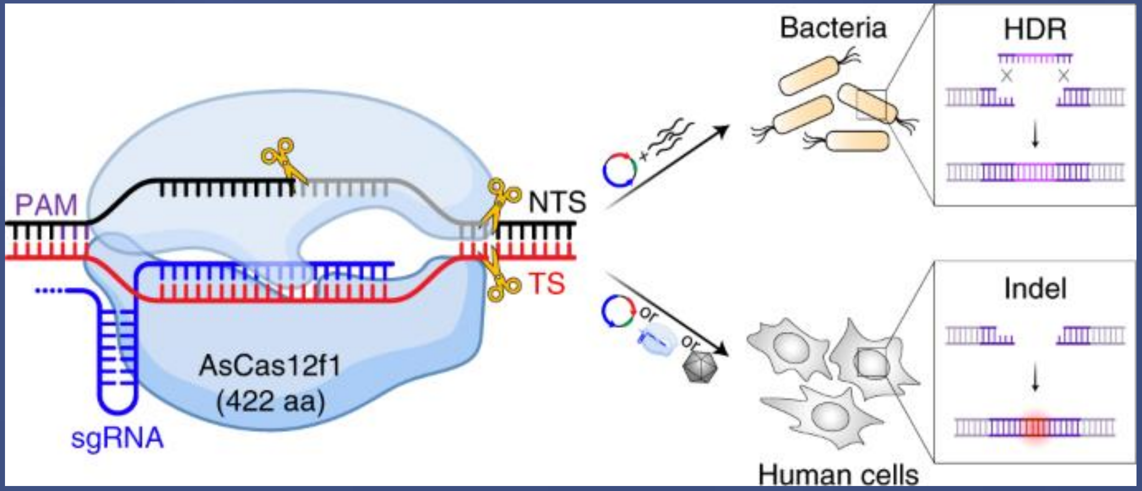
New Tools

Bigelyte, Greta, … Gasior, Stephen…et al. "Miniature type VF CRISPR-Cas nucleases enable targeted DNA modification in cells." *Nature communications* 12.1 (2021): 1-8.

Xu, Xiaoshu, et al. "Engineered miniature CRISPR-Cas system for mammalian genome regulation and editing." *Molecular Cell* 81.20 (2021): 4333-4345.

Wu, Zhaowei, et al. "Programmed genome editing by a miniature CRISPR-Cas12f nuclease." *Nature Chemical Biology* 17.11 (2021): 1132-1138.

Kim, Do Yon, et al. "Efficient CRISPR editing with a hypercompact Cas12f1 and engineered guide RNAs delivered by adeno-associated virus." *Nature Biotechnology* (2021): 1-9.



Smaller editing Cas proteins enable delivery on AAV

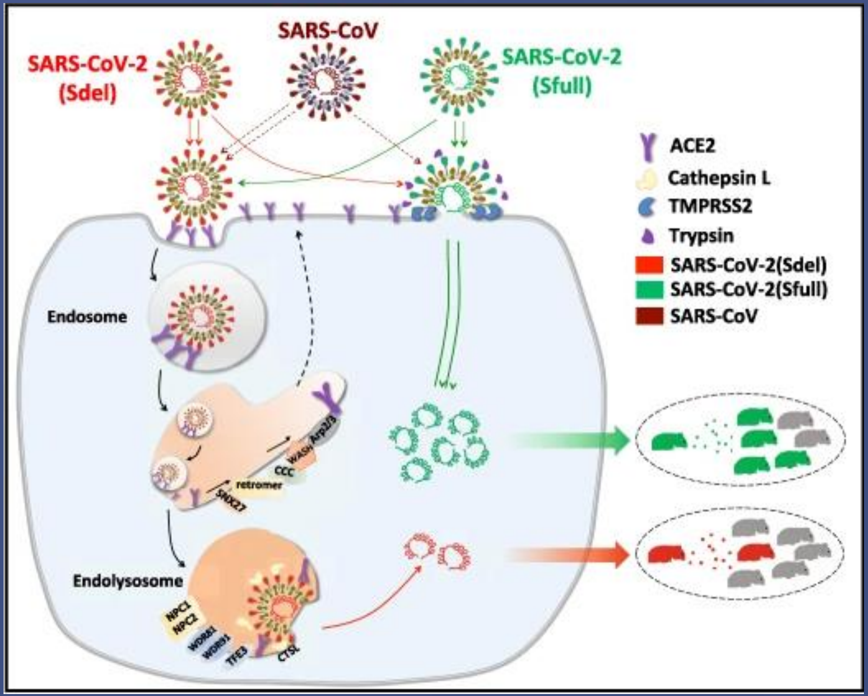
Also, new IP landscape

By comparison, Cas9 is 1500 aa

New Tools

"A genome-wide CRISPR screen identifies host factors that regulate SARS-CoV-2 entry"

Genome screening technology – what promotes the survival of cells in face of infection?

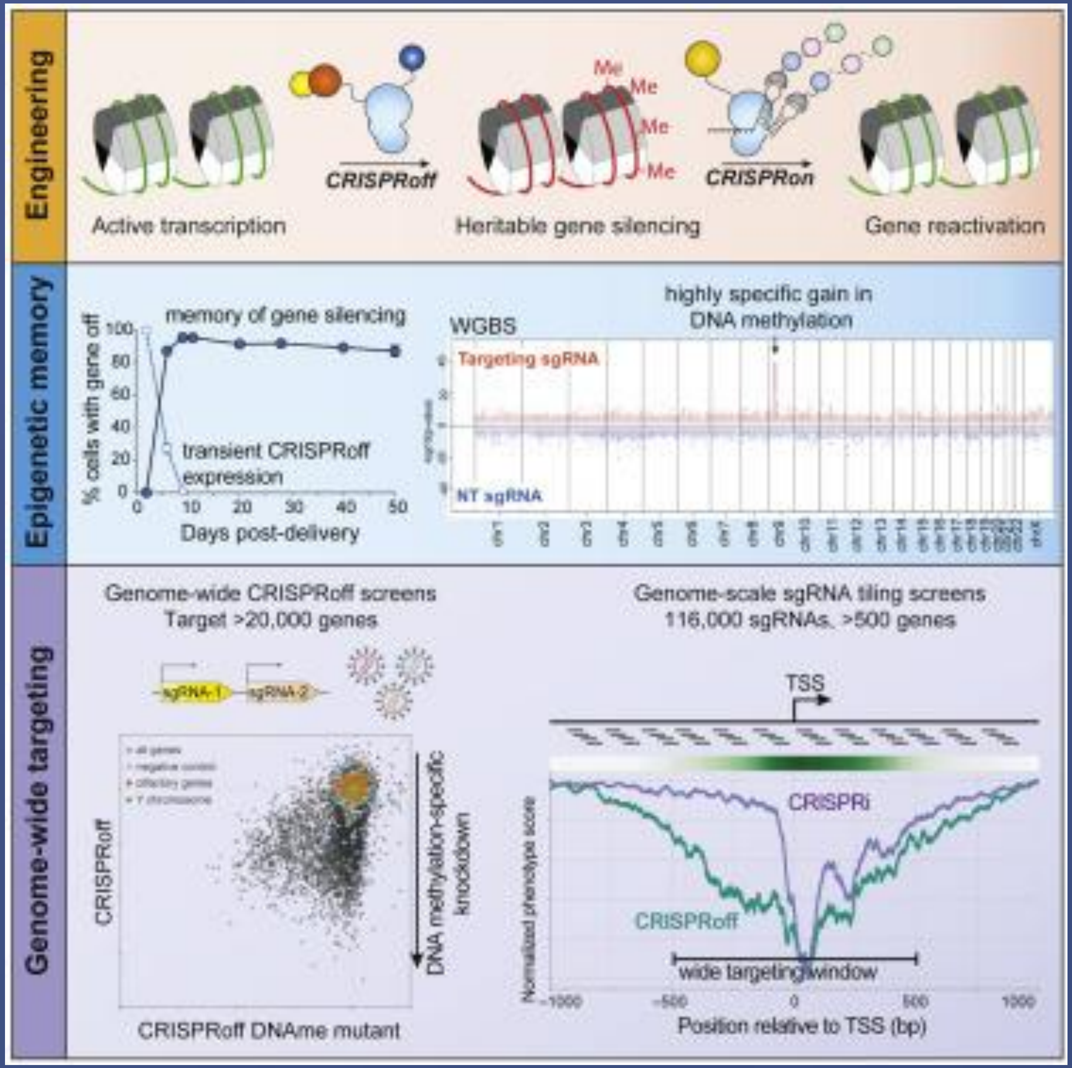


Found genes that promote the endosomal pathway aided by ACE expression. Virus can be retransmitted

Zhu, Yunkai, et al. *Nature communications* 12.1 (2021): 1-11.

New Tools

"Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing"



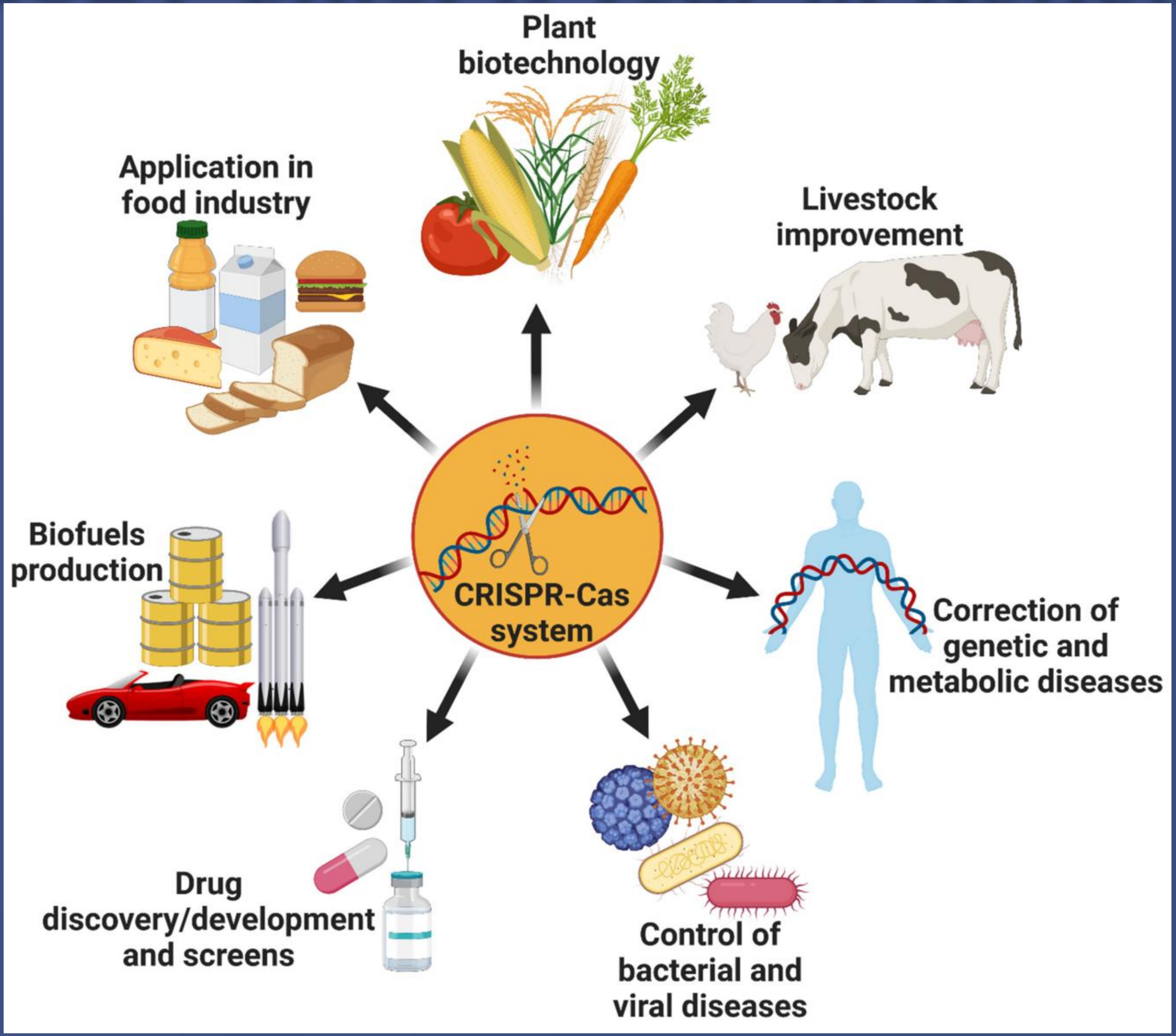
"dead" Cas9 with transcription activators and silencers demonstrated with multiple gene targets and inheritable in inducible pluripotent stem cells.

Applications include tissue engineering

Applications also include pharmacology

XNuñez, James K., et al. *Cell* 184.9 (2021): 2503-2519.

2021 CRISPR Year in Review



Nidhi, Sweta, et al. "Novel CRISPR-Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives." *International Journal of Molecular Sciences* 22.7 (2021): 3327.

2021 CRISPR Year in Review

A very exciting year demonstrating the wide range of genome editing techniques and applications.

Questions?