2023 Genome Editing Year in Review

Science Circle
January 6th 2024

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Scientist
Corteva Agriscience

2023 Genome Engineering Year in Review

Background

Therapies/To the Consumer Advances

Delivery Advances

Target Genes and Modified Organisms

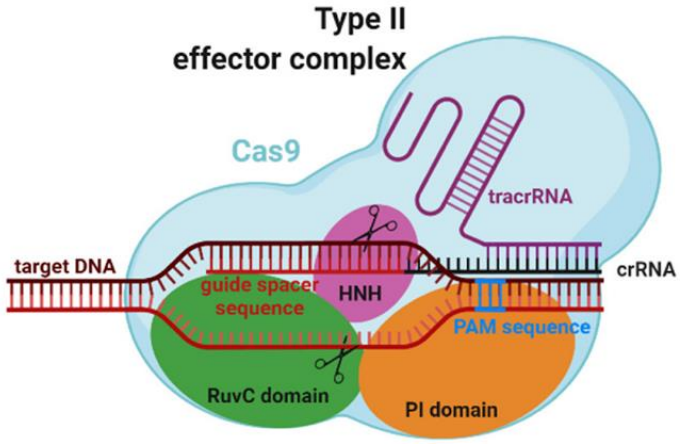
New Tools

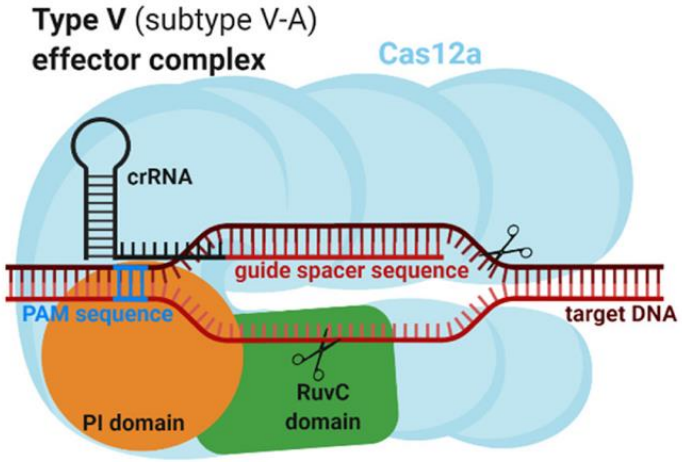
Cautionary Tales

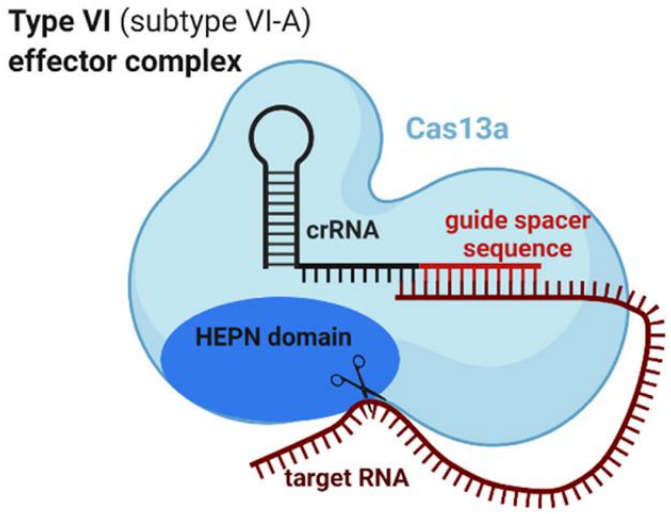
Researcher at Corteva. AgBioTech that operates in this space. Not representing the company's positions.

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

2023 Genome Engineering Year in Review







Cas9 First and most studied and used

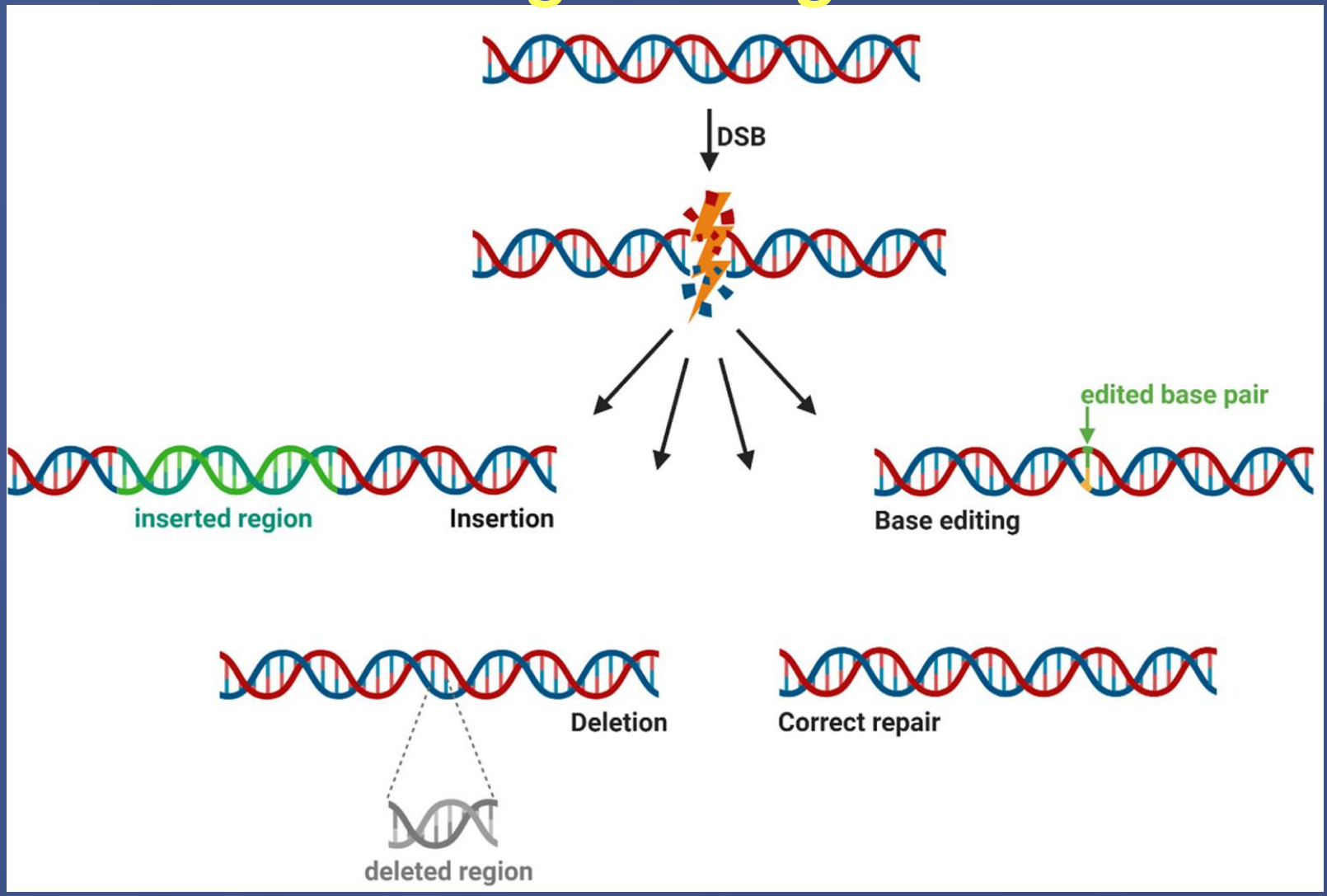
Cas12a Second most well studied (large families of Cas12s)

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.

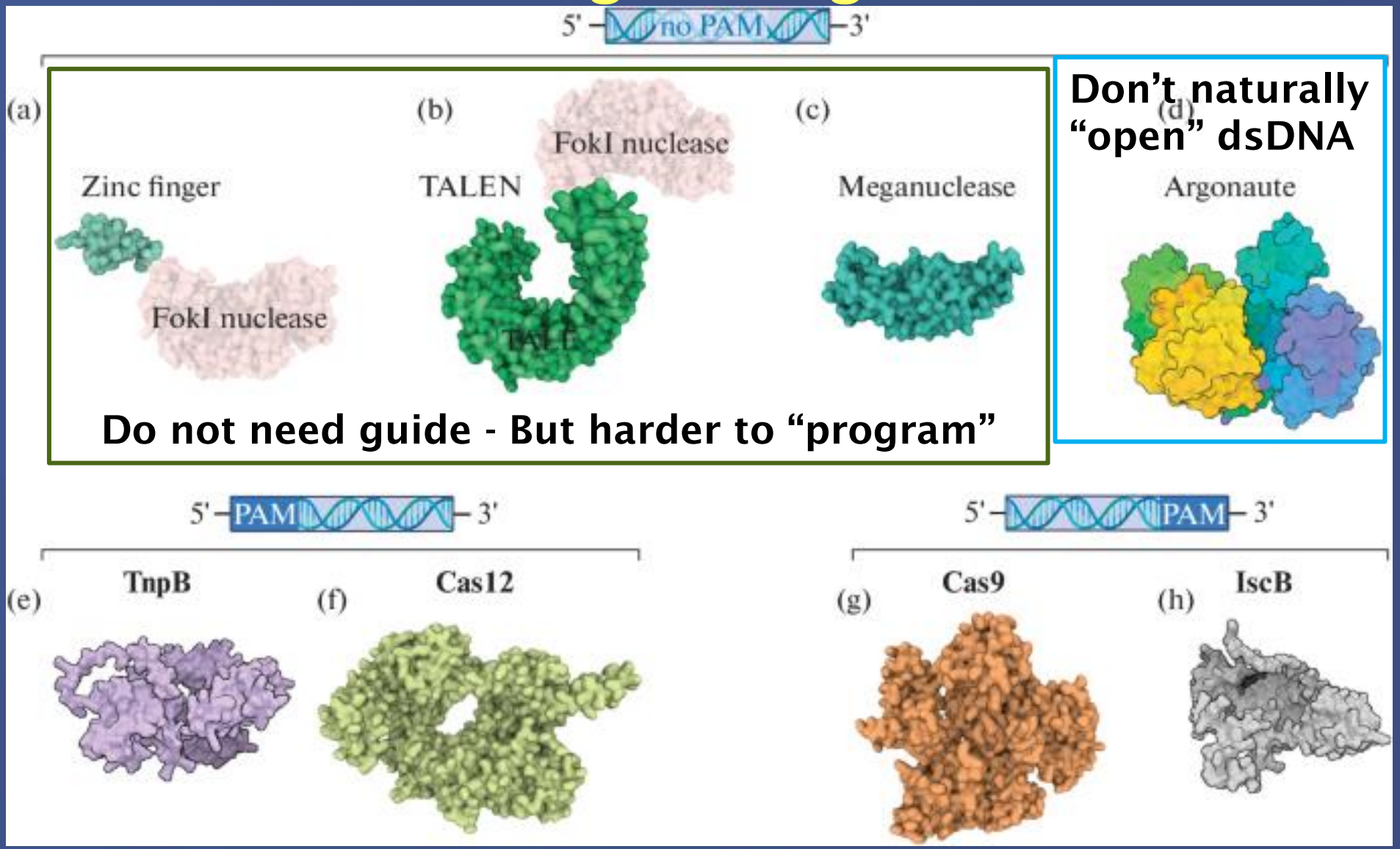
2023 Genome Engineering Year in Review



A double strand break can modify or add sequences at a target location. A single-strand break and a base editor can edit a base. DSBs are still very dangerous.

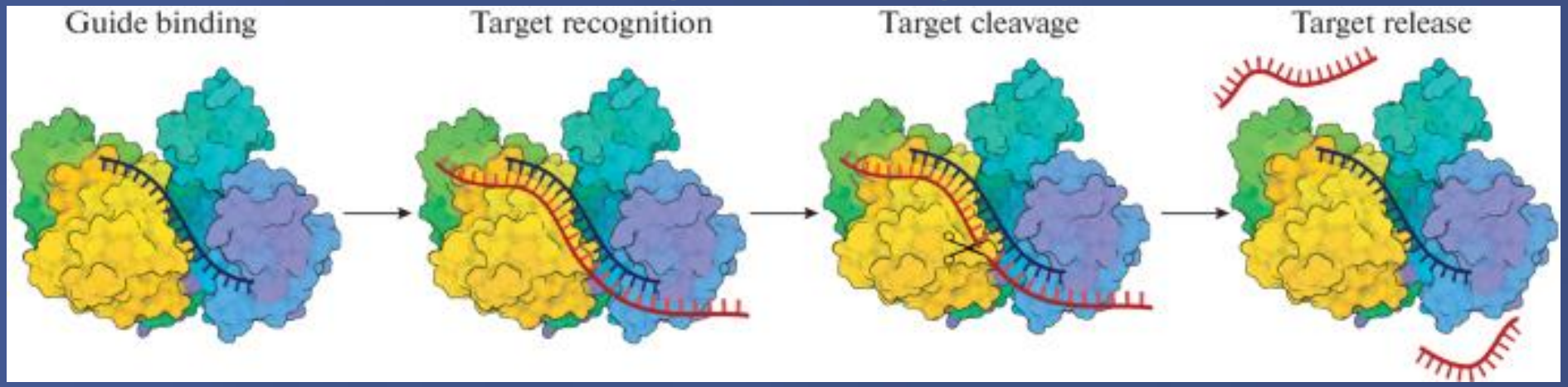
Nidhi, Sweta, et al. "Novel CRISPR–Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives." *International Journal of Molecular Sciences* 22.7 (2021): 3327.

2023 Genome Engineering Year in Review



Kropocheva, E. V., et al. "Prokaryotic Argonaute Proteins as a Tool for Biotechnology." *Molecular Biology* (2022): 1-20.

2023 Genome Engineering Year in Review



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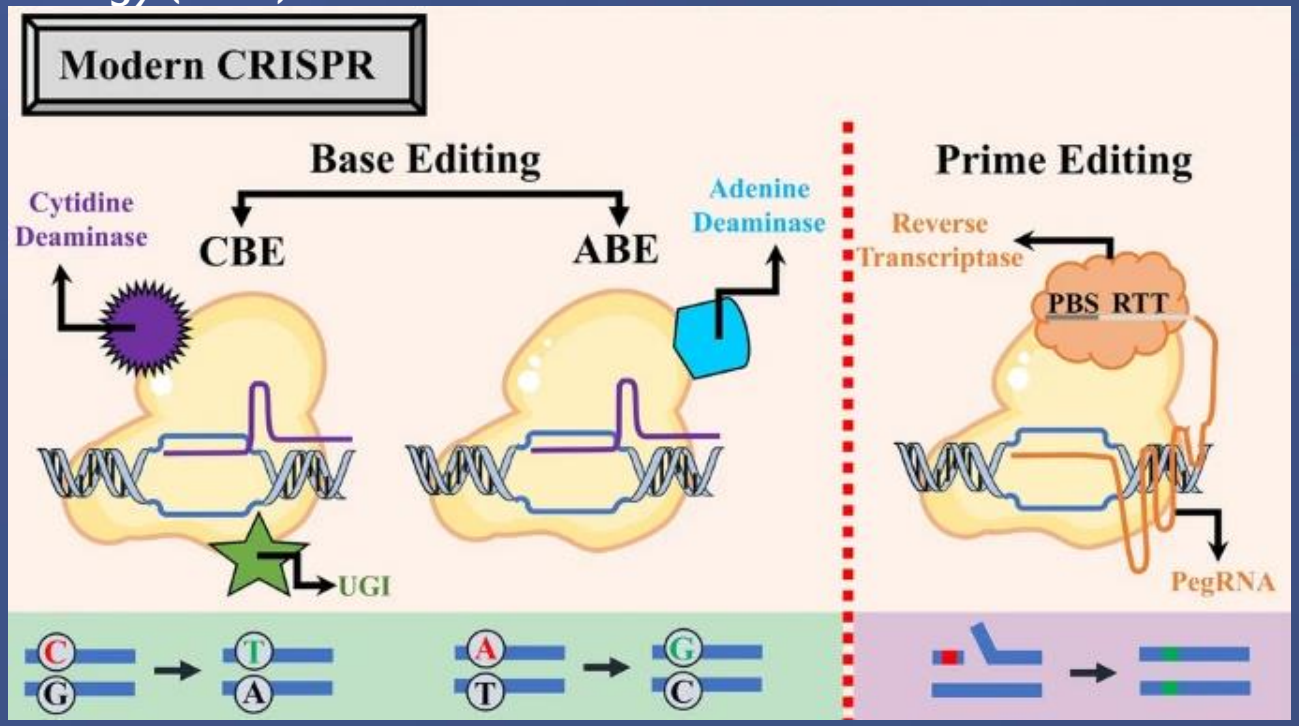


Saber Sichani, Ali, et al. "A Review on Advanced CRISPR-Based Genome-Editing Tools: Base Editing and Prime Editing." *Molecular Biotechnology* (2022): 1-12.

Insert novel DNA without a double-strand break

Therapy Advances

UK first to approve CRISPR treatment for diseases: what you need to know

https://www.nature.com/articles/d41586-023-03590-6

-The therapy, called Casgevy, will treat the blood conditions sickle-cell disease and β-thalassaemia. Sickle-cell disease, also known as sickle-cell anaemia, can cause debilitating pain, and people with β-thalassaemia often require regular blood transfusions.

-The therapy was developed by the pharmaceutical company Vertex Pharmaceuticals in Boston, Massachusetts, and biotechnology company CRISPR Therapeutics in Zug, Switzerland

-The trial for sickle-cell disease has followed 29 out of 45 participants long enough to draw interim results. Casgevy completely relieved 28 of those people of debilitating episodes of pain for at least one year after treatment.

-Among 42 severe form of β-thalassaemia participants, 39 did not need a red-blood-cell transfusion for at least one year.

Therapy Advances

Verve Therapeutics Announces Interim Data for VERVE-101 Demonstrating First Human Proof-of-Concept for In Vivo Base Editing with Dose-Dependent Reductions in LDL-C and Blood PCSK9 Protein in Patients with Heterozygous Familial Hypercholesterolemia

https://ir.vervetx.com/news-releases/news-release-details/verve-therapeutics-announces-interim-data-verve-101

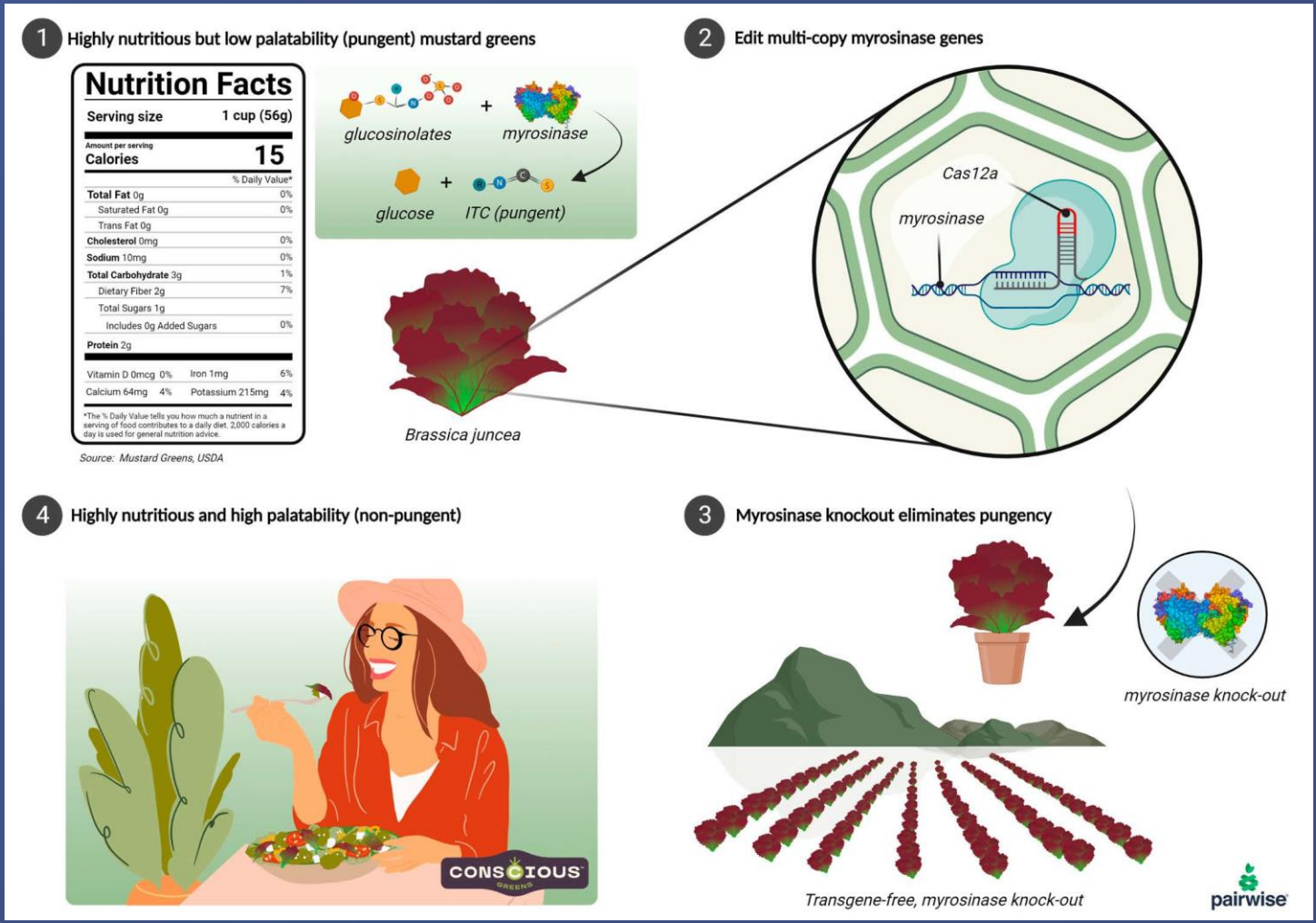
-LDL-C Reductions Up to 55% and Blood PCSK9 Protein Reductions Up to 84% Observed After a Single Infusion of VERVE-101 at Potentially Therapeutic Doses

-In this single participant in the highest dose cohort, the 55% reduction in LDL-C was durable out to 180 days, with follow-up ongoing.

Edited plants hit US produce shelves

Pairwise Introduces Conscious Greens into U.S. Restaurants

https://www.seedworld.com/pairwise-introduces-conscious-greens-into-u-s-restaurants/

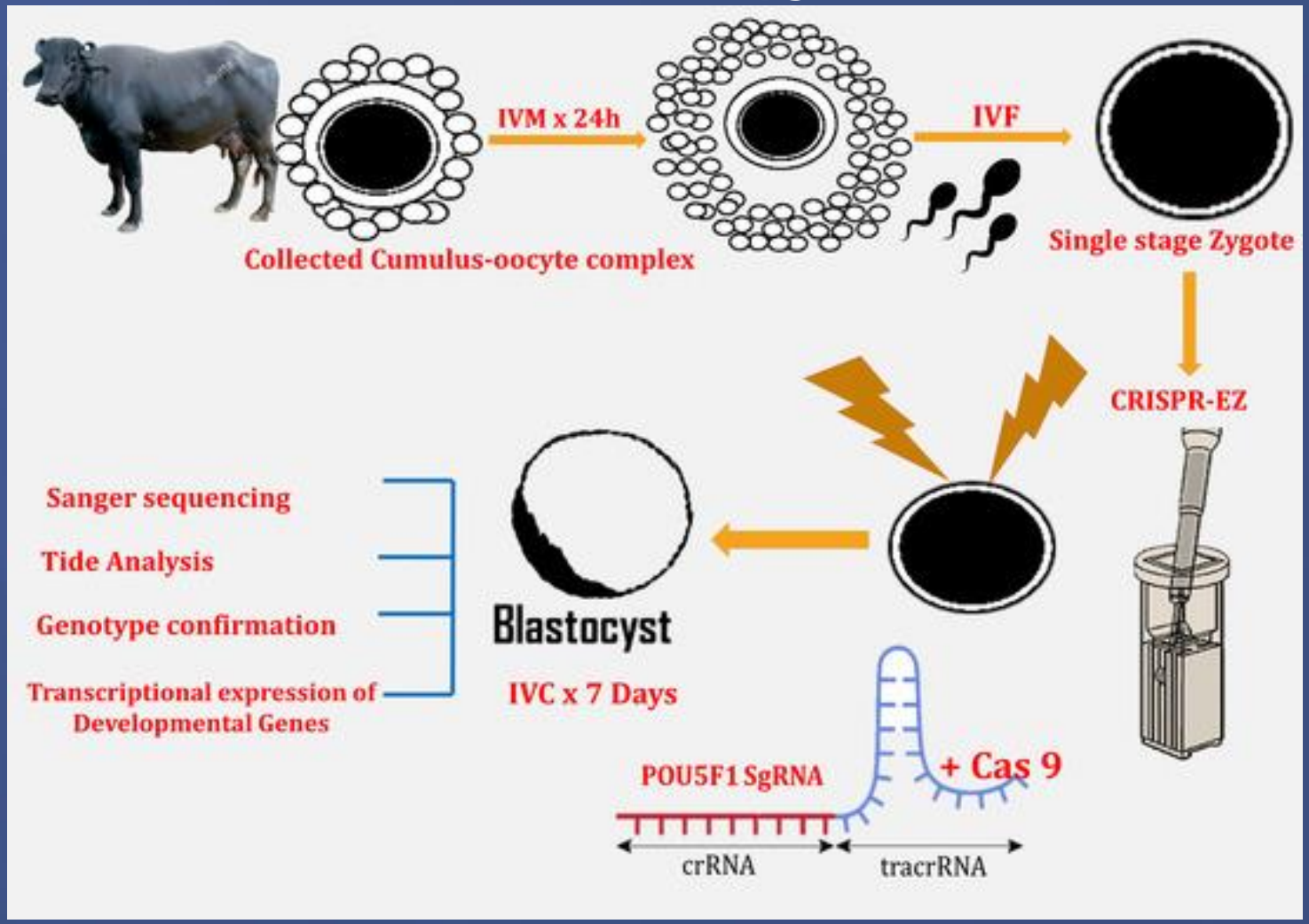


Karlson, Dale, et al. "Targeted Mutagenesis of the Multicopy Myrosinase Gene Family in Allotetraploid *Brassica juncea* Reduces Pungency in Fresh Leaves across Environments." *Plants* 11.19 (2022): 2494.

https://www.pairwise.com/conscious-foods

Delivery Advances

Punetha, Meeti, et al. "Optimising Electroporation Condition for CRISPR/Cas-Mediated Knockout in Zona-Intact Buffalo Zygotes." *Animals* 14.1 (2023): 134.

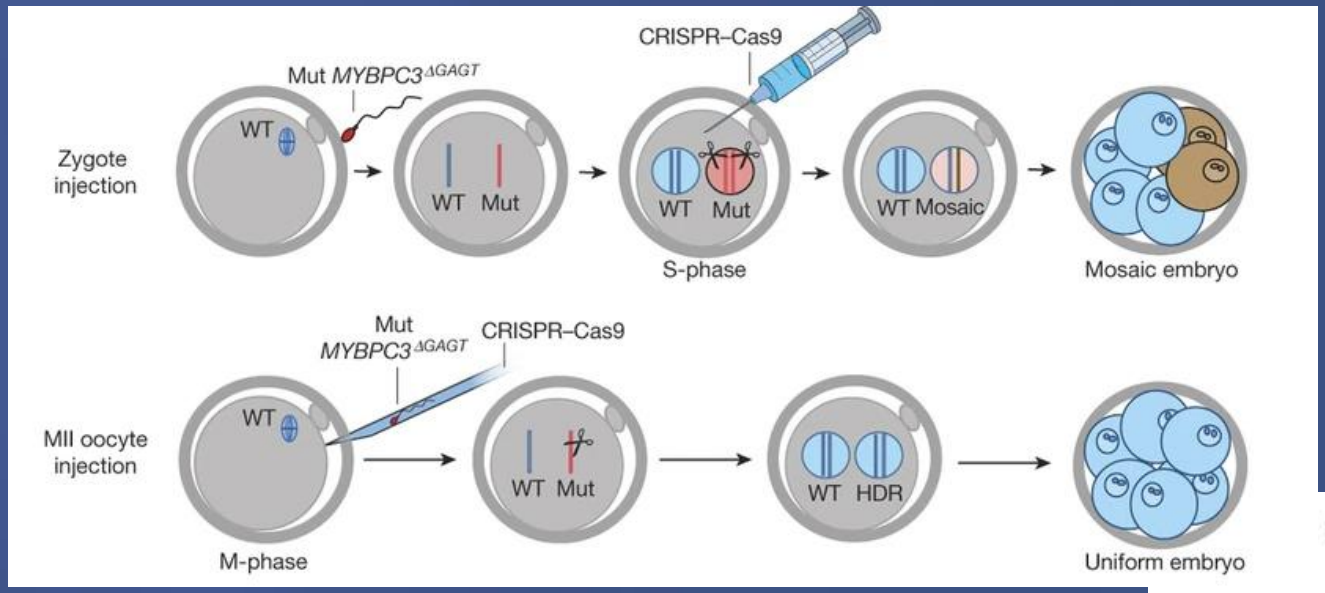


Enables efficient genome editing even outside of model organisms

Limited to mutations

Delivery Advances

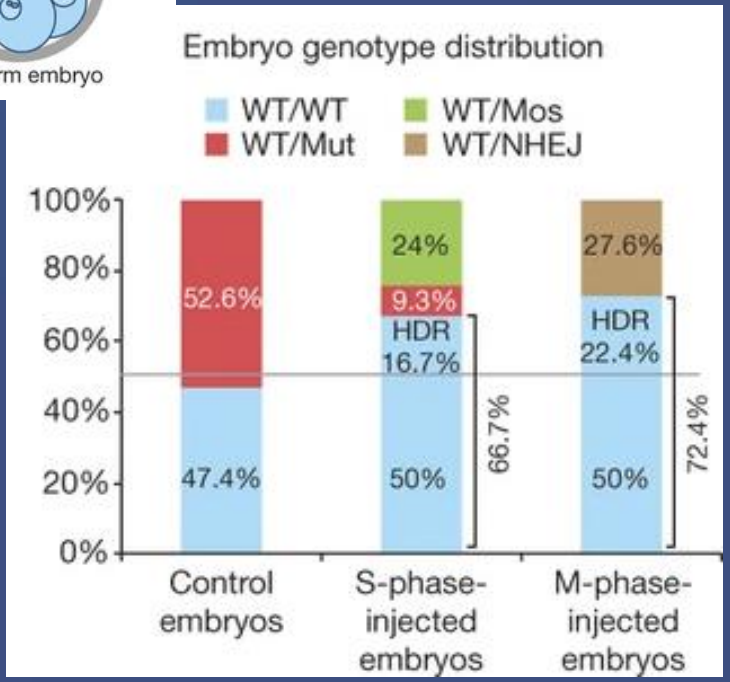
Ma, Hong, et al. "Correction of a pathogenic gene mutation in human embryos." *Nature* 548.7668 (2017): 413-419.



Timing the delivery to BEFORE the first DNA replication, reduces mosaicism.

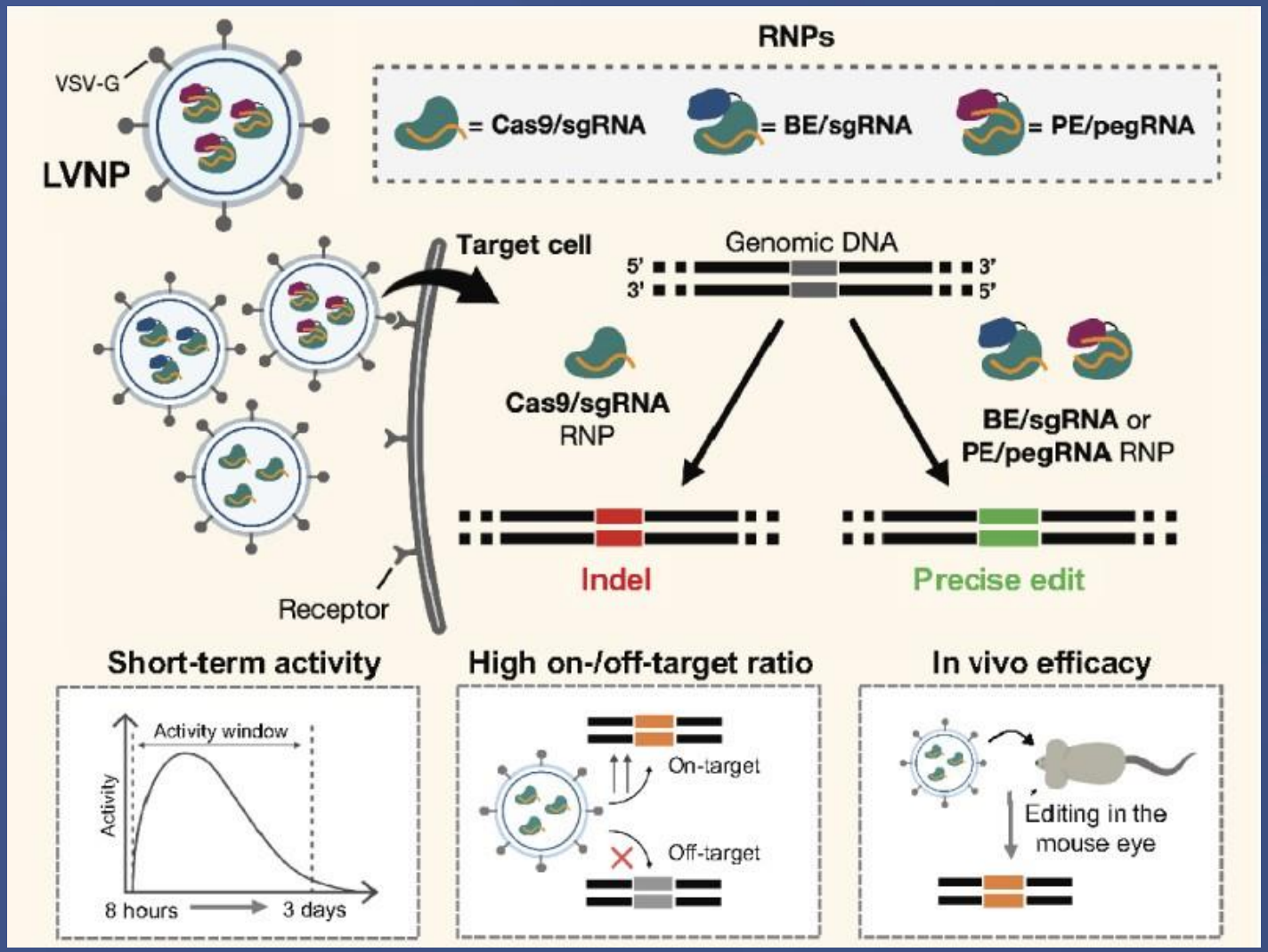
Co-injecting with sperm works even better.

HR preferred in this cell context



Delivery Advances

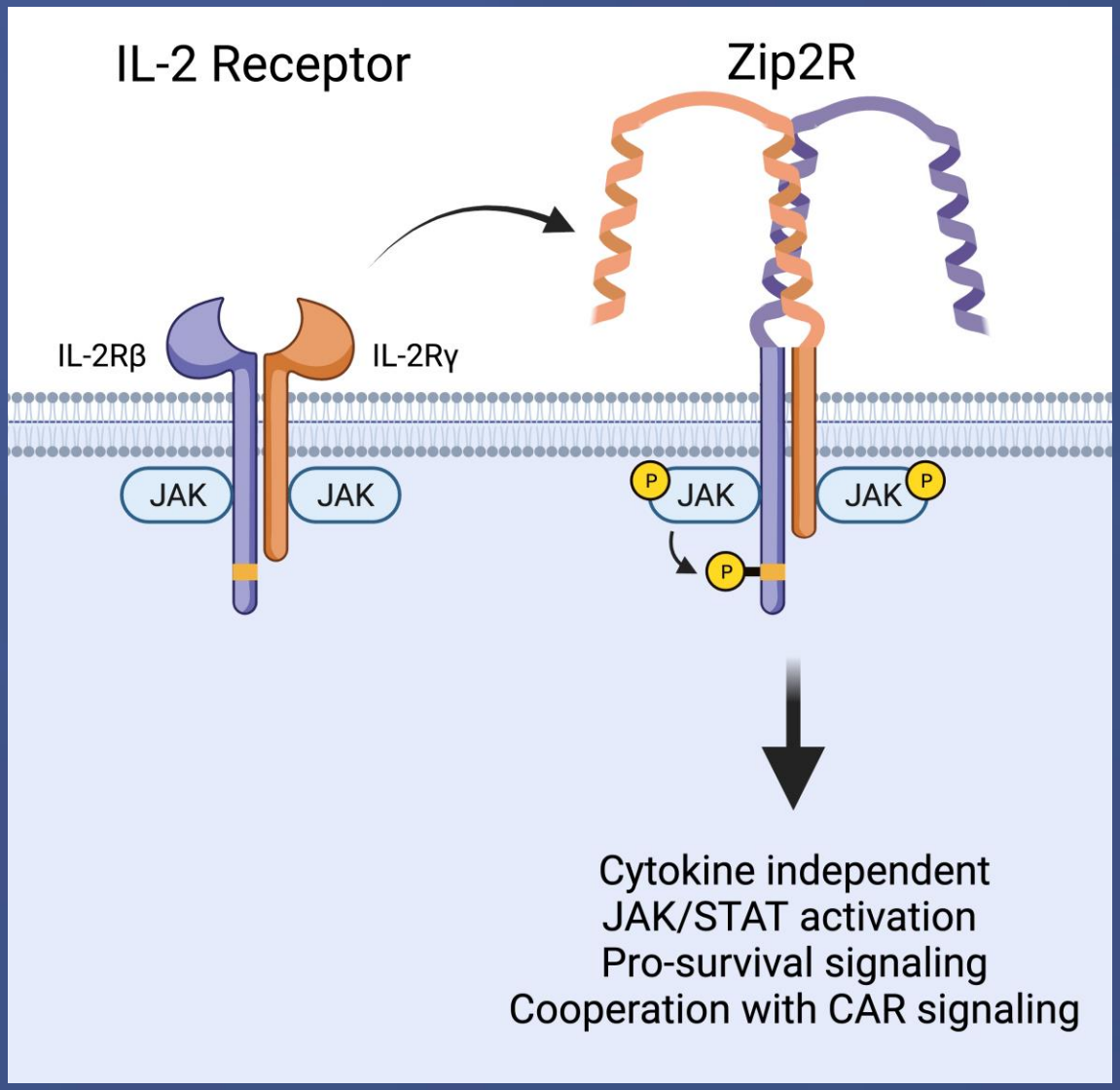
Haldrup, Jakob, et al. "Engineered lentivirus-derived nanoparticles (LVNPs) for delivery of CRISPR/Cas ribonucleoprotein complexes supporting base editing, prime editing and in vivo gene modification." *Nucleic Acids Research* 51.18 (2023): 10059-10074.



"in the mouse eye, we provide the first proof-of-concept for LVNP-directed *in vivo* gene disruption."

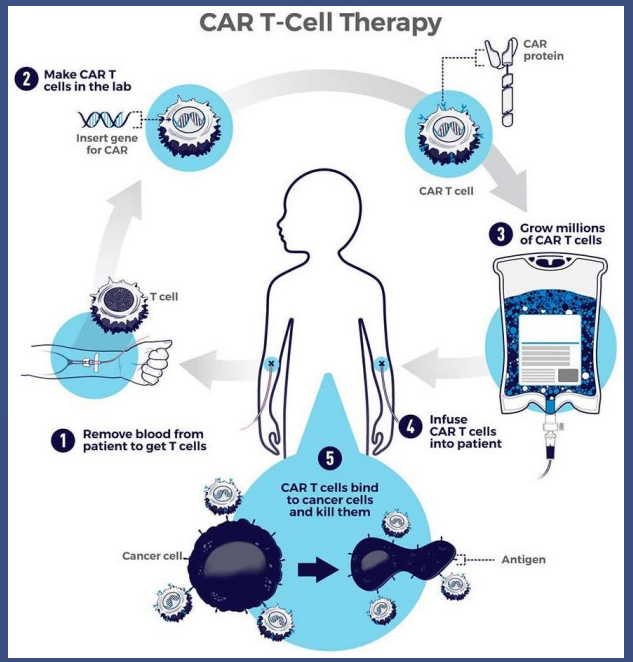
Delivery Advances

Bell, Matthew, et al. "Modular chimeric cytokine receptors with leucine zippers enhance the antitumour activity of CAR T cells via JAK/STAT signaling." *Nature Biomedical Engineering* (2023): 1-17.



Enables CAR-T therapy to target SOLID tumors

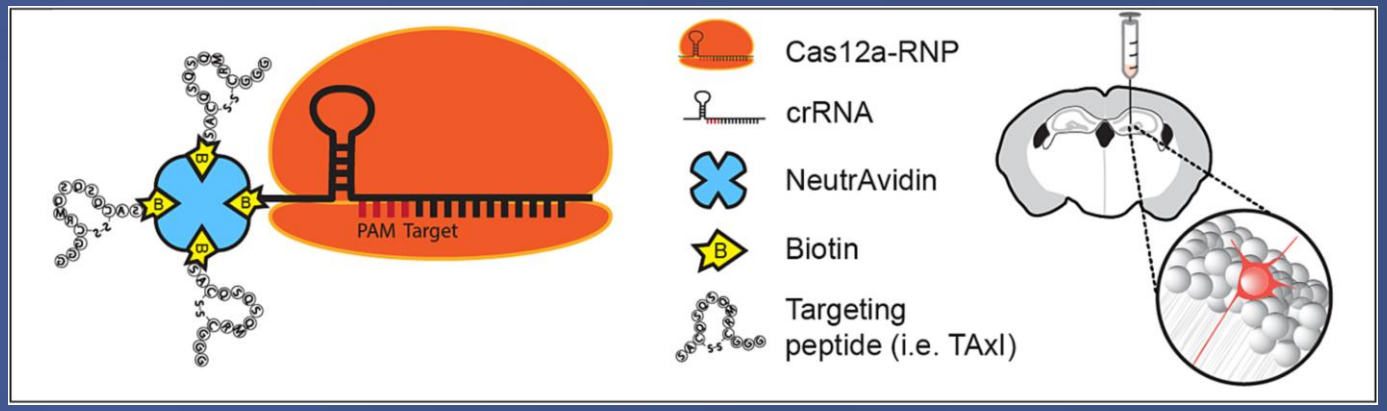
reminder



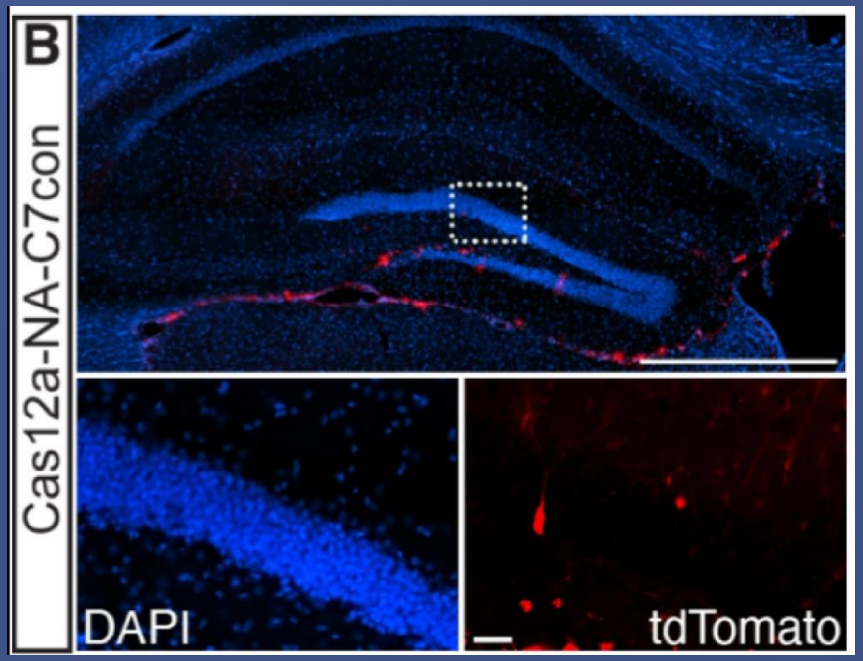
https://www.cancer.gov/about-cancer/treatment/research/car-t-cells

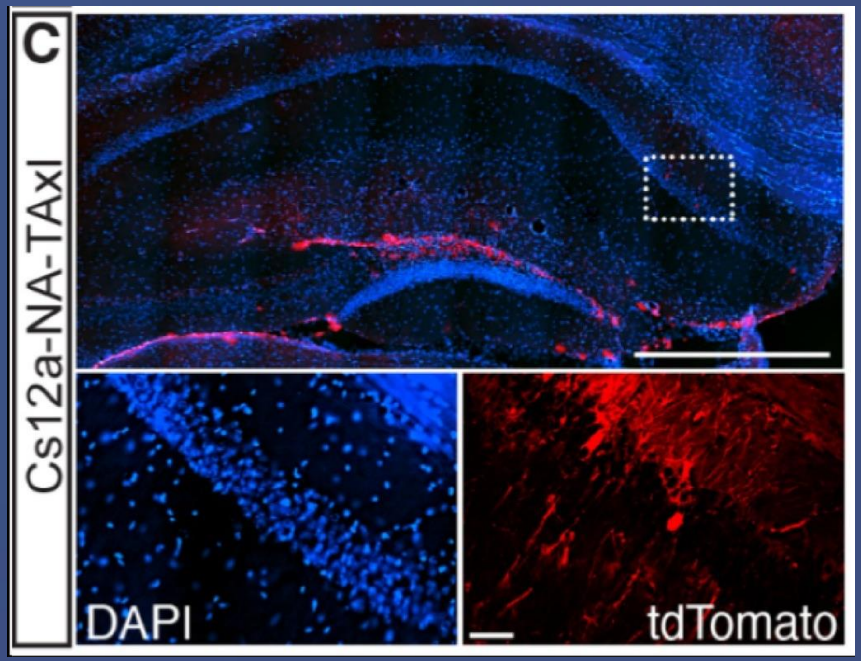
Delivery Advances

Sellers, Drew L., et al. "TAxI-peptide targeted Cas12a ribonuclease protein nanoformulations increase genome editing in hippocampal neurons." *Journal of Controlled Release* 354 (2023): 188-195.



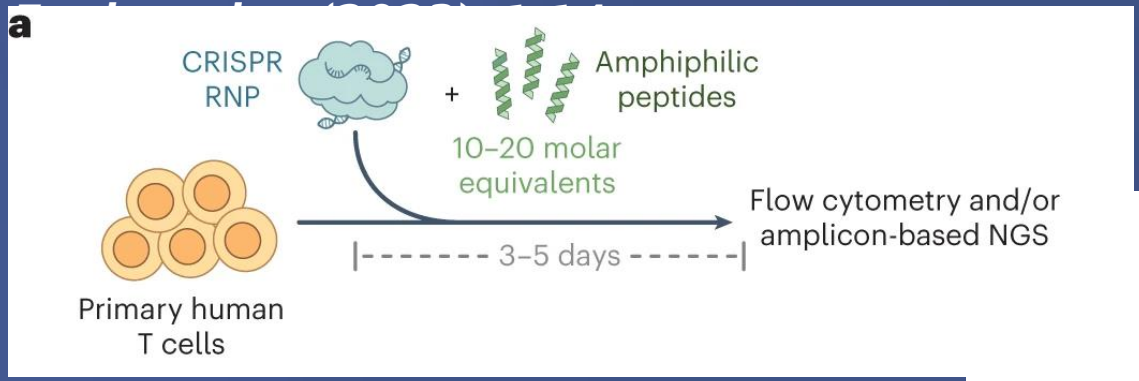
Targeting specific brain cells (mice)

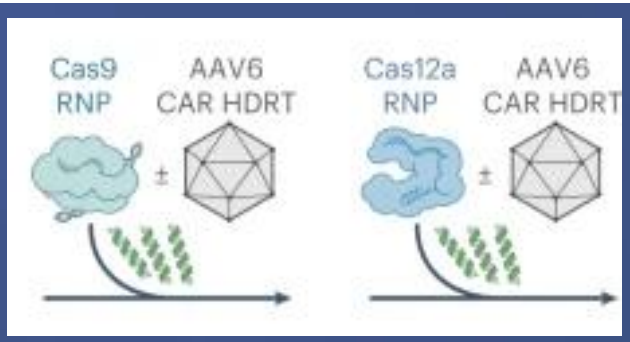


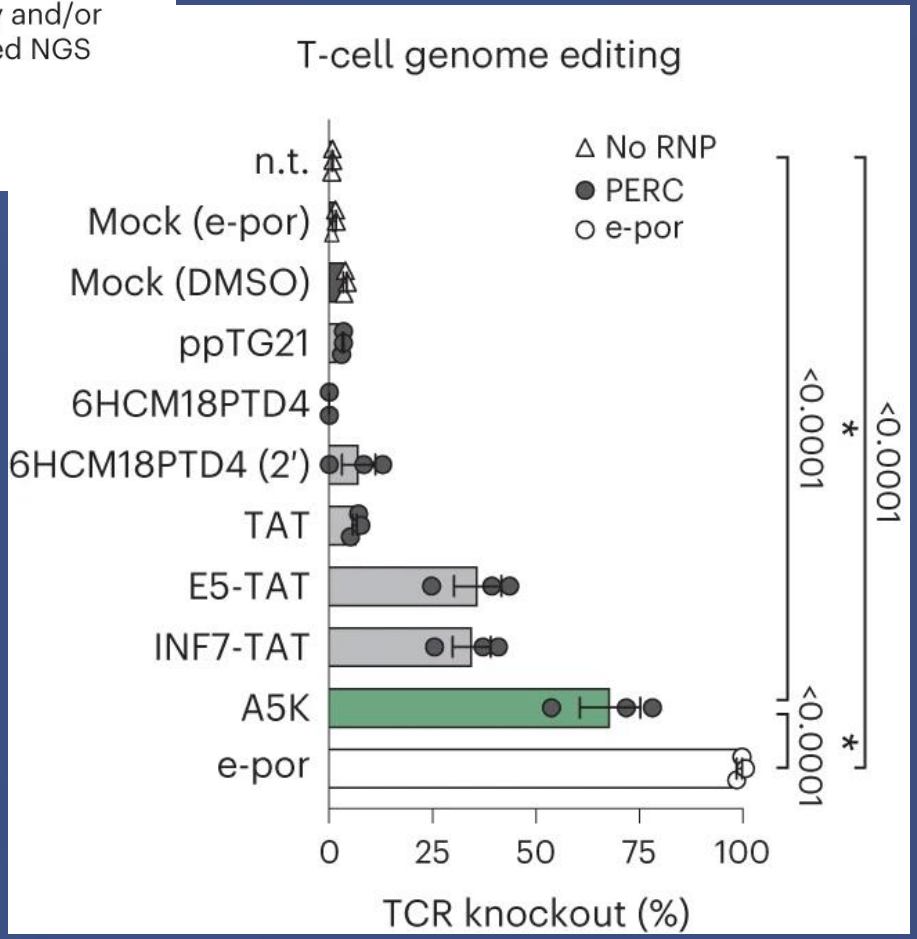


Delivery Advances

Foss, Dana V., et al. "Peptide-mediated delivery of CRISPR enzymes for the efficient editing of primary human lymphocytes." *Nature Biomedical*





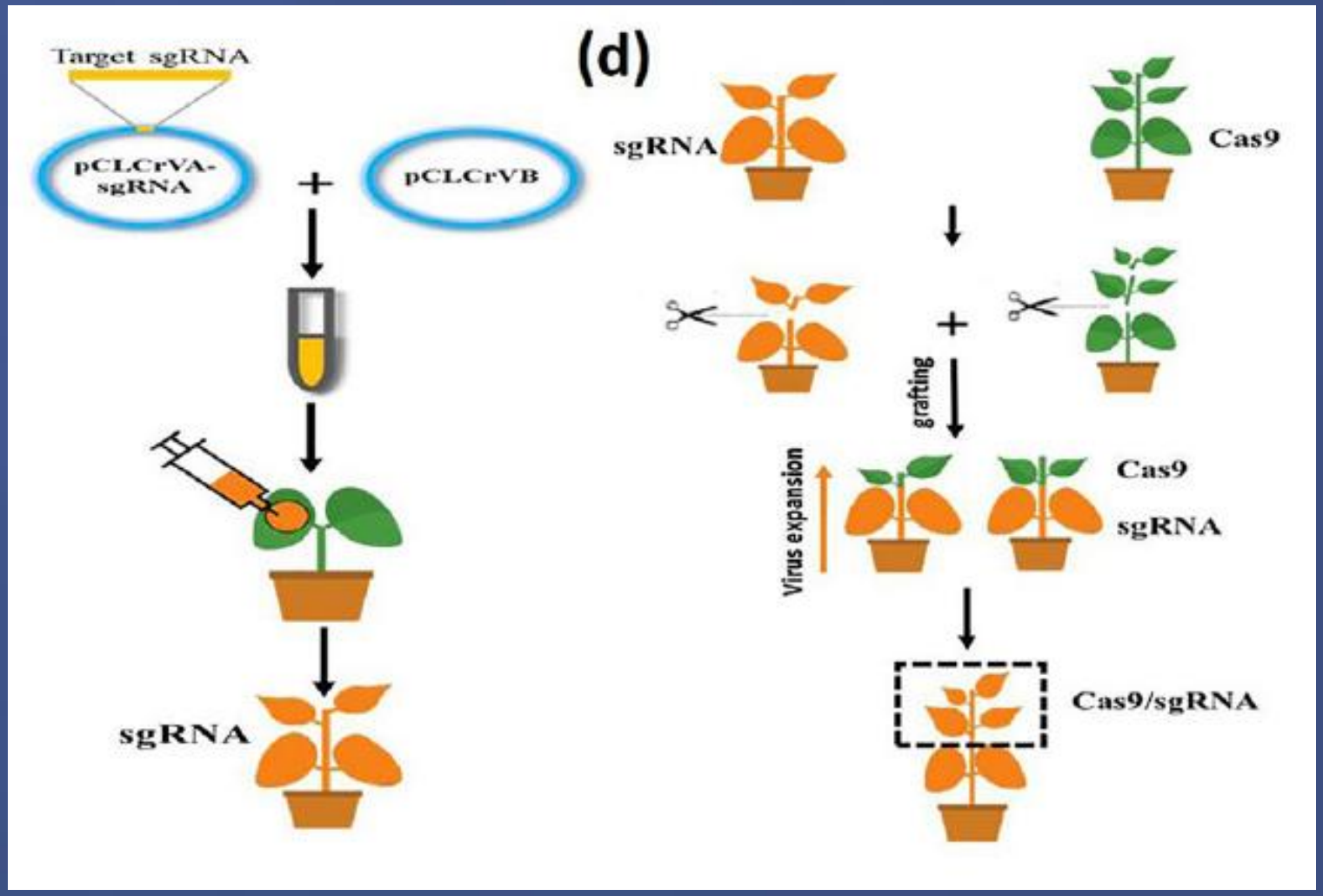


This can be used for direct in-patient CAR-T therapy potentially

Includes HR template in AAV vector

Delivery Advances

Guo, Wei-Feng, et al. "Efficient genome editing in cotton using the virus-mediated CRISPR/Cas9 and grafting system." *Plant Cell Reports* 42.11 (2023): 1833-1836.

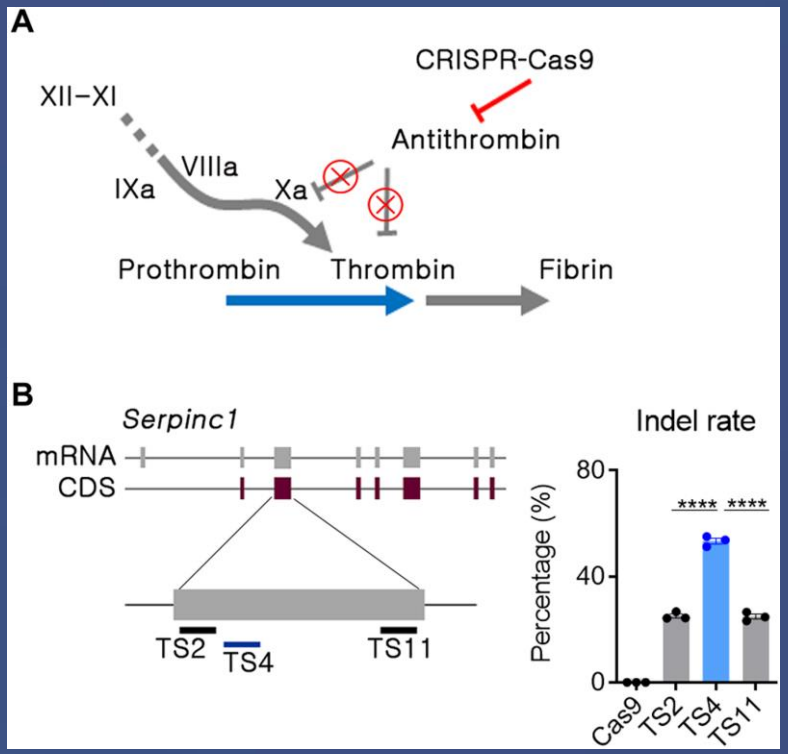


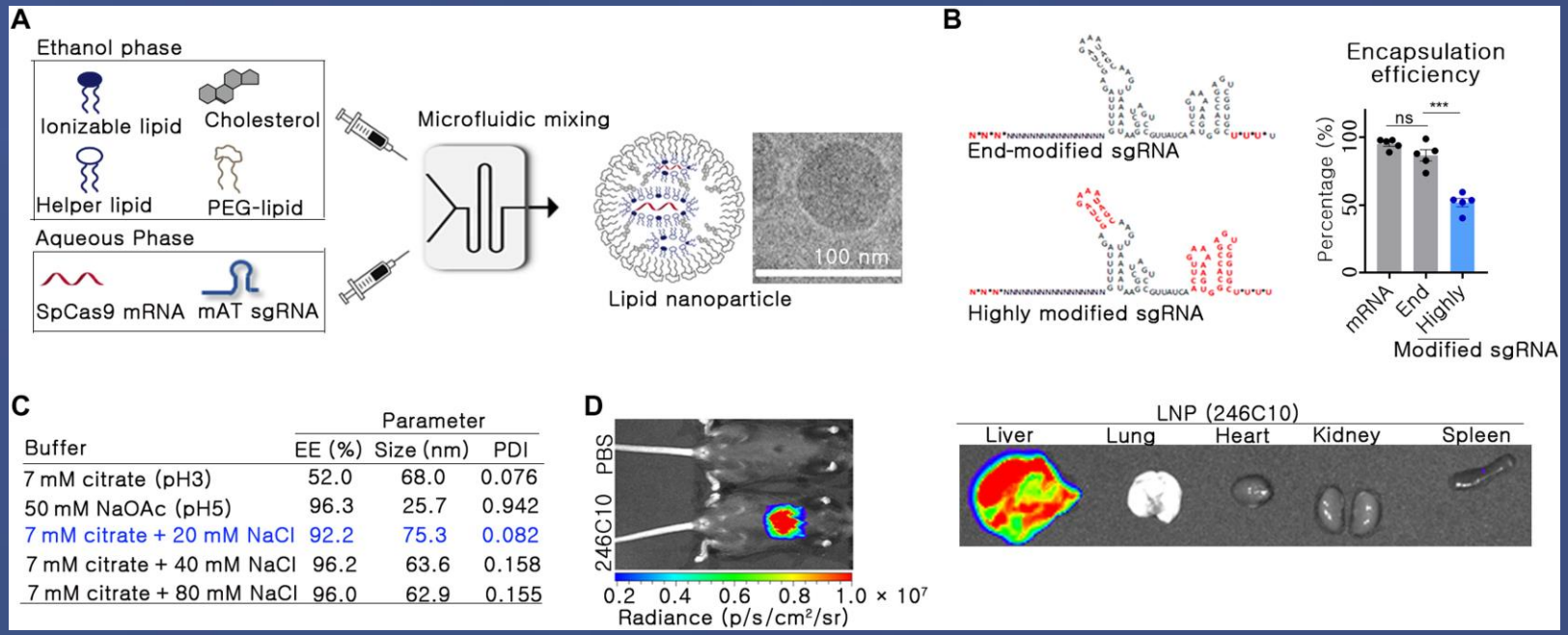
Guide travels via RNA virus to parts that have Cas9 cassette

Target Genes and Modified Organisms

Han, Jeong Pil, et al. "*In vivo* delivery of CRISPR-Cas9 using lipid nanoparticles enables antithrombin gene editing for sustainable hemophilia A and B therapy." *Science advances* 8.3 (2022): eabj6901.

Another "one-shot" therapy for sustained treatment. (mice)



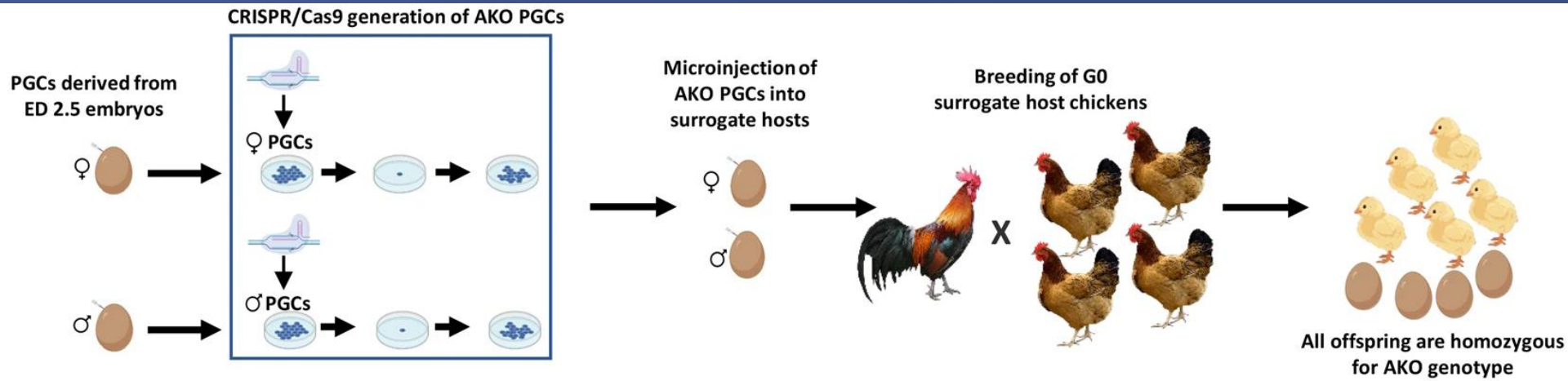


C

Buffer	Parameter		
	EE (%)	Size (nm)	PDI
7 mM citrate (pH3)	52.0	68.0	0.076
50 mM NaOAc (pH5)	96.3	25.7	0.942
7 mM citrate + 20 mM NaCl	92.2	75.3	0.082
7 mM citrate + 40 mM NaCl	96.2	63.6	0.158
7 mM citrate + 80 mM NaCl	96.0	62.9	0.155

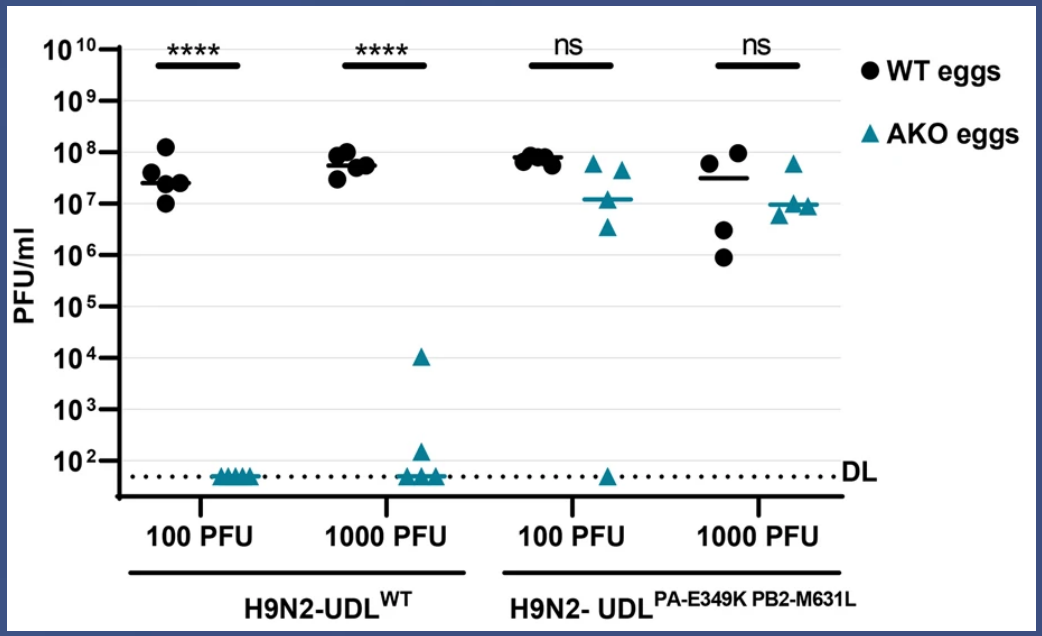
Target Genes and Modified Organisms

Idoko-Akoh, Alewo, et al. "Creating resistance to avian influenza infection through genome editing of the ANP32 gene family." *Nature Communications* 14.1 (2023): 6136.



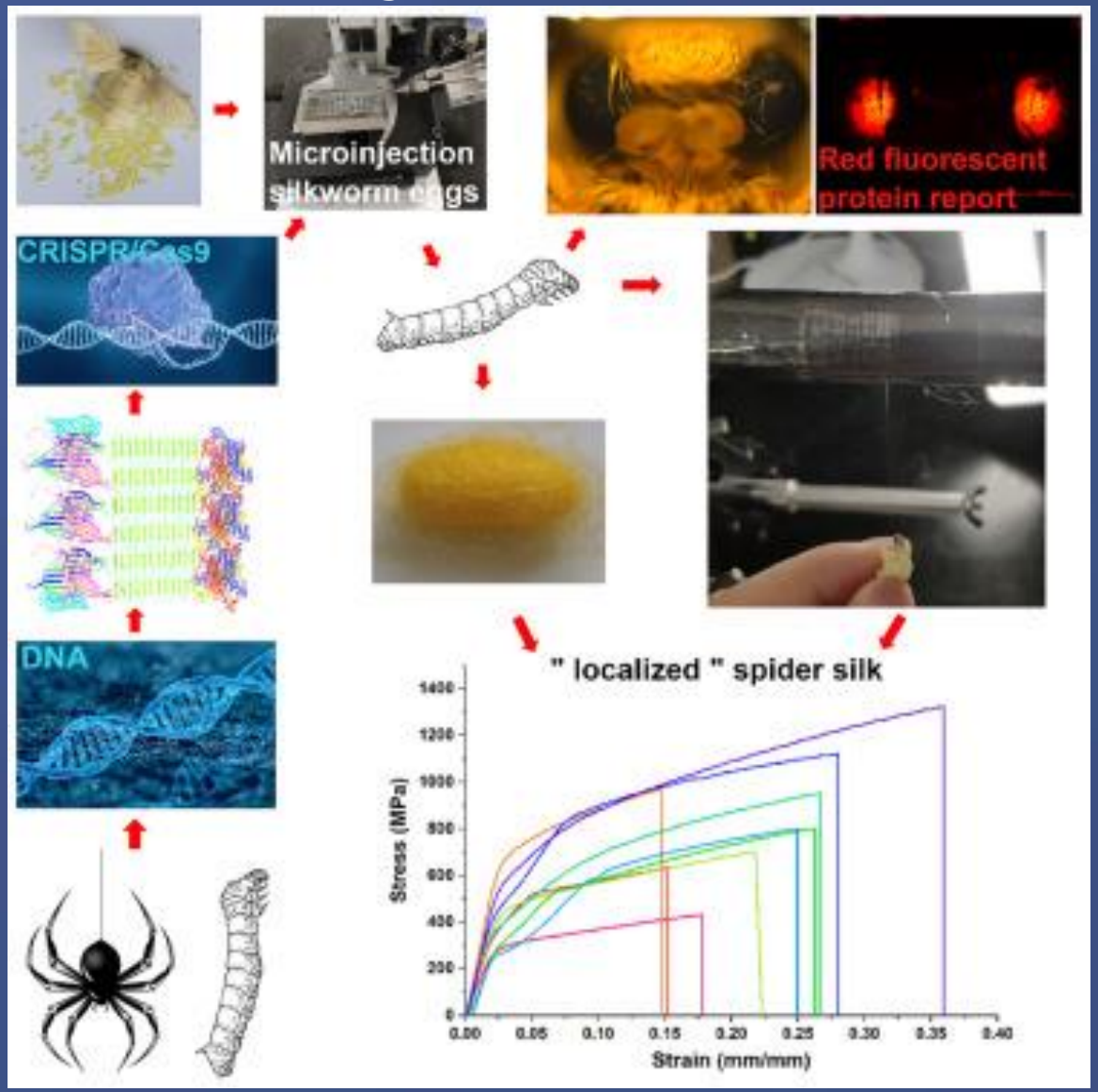
Removing an important host factor necessary for viral replication makes them immune and unable to pass the virus along

Prophylactic strategy for livestock



Target Genes and Modified Organisms

Mi, Junpeng, et al. "High-strength and ultra-tough whole spider silk fibers spun from transgenic silkworms." *Matter* 6.10 (2023): 3661-3683.

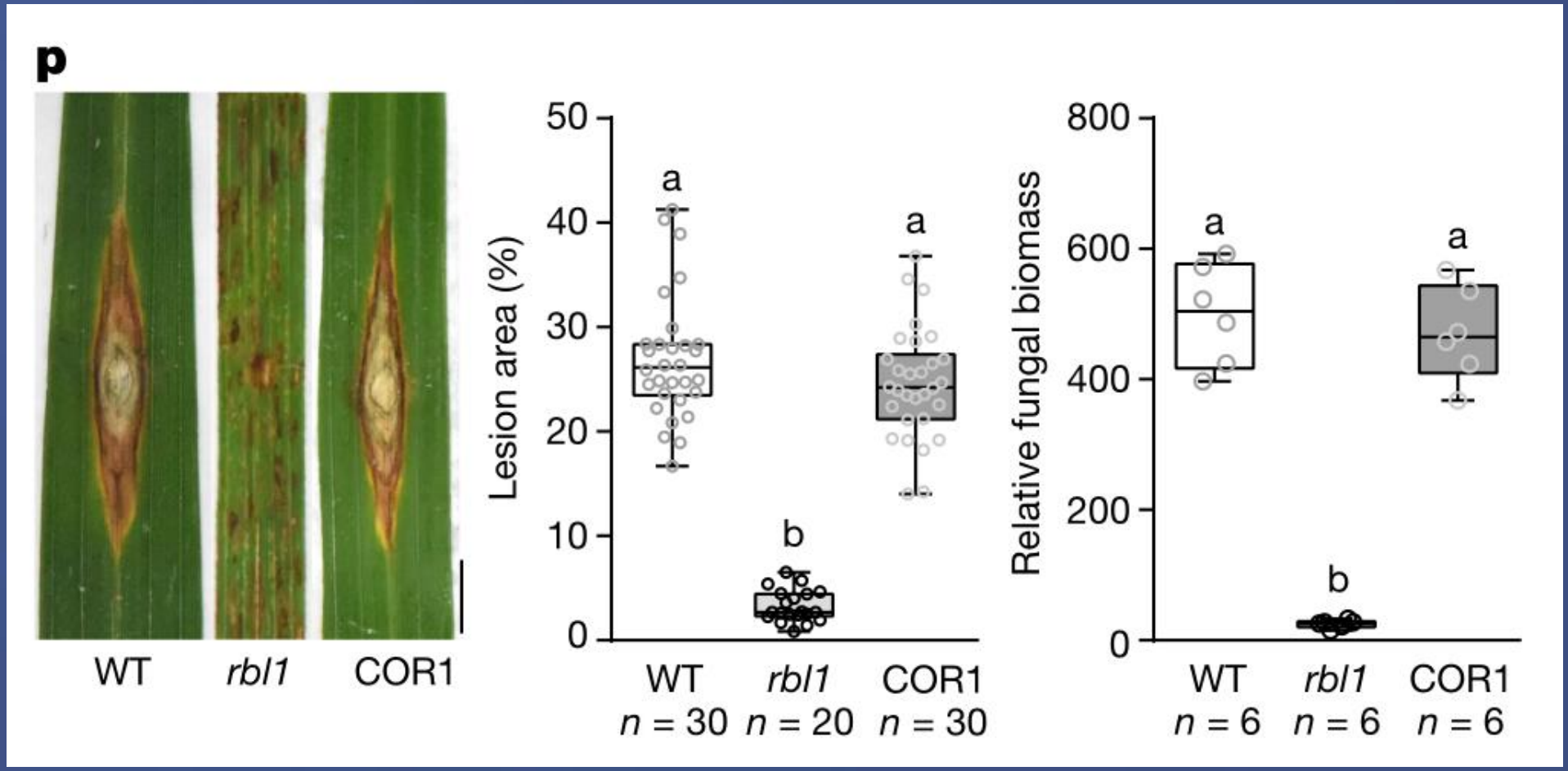


CRISPR enables “species transfer” of a produced biomaterial.

Also helps inform the chemistry of its production

Target Genes and Modified Organisms

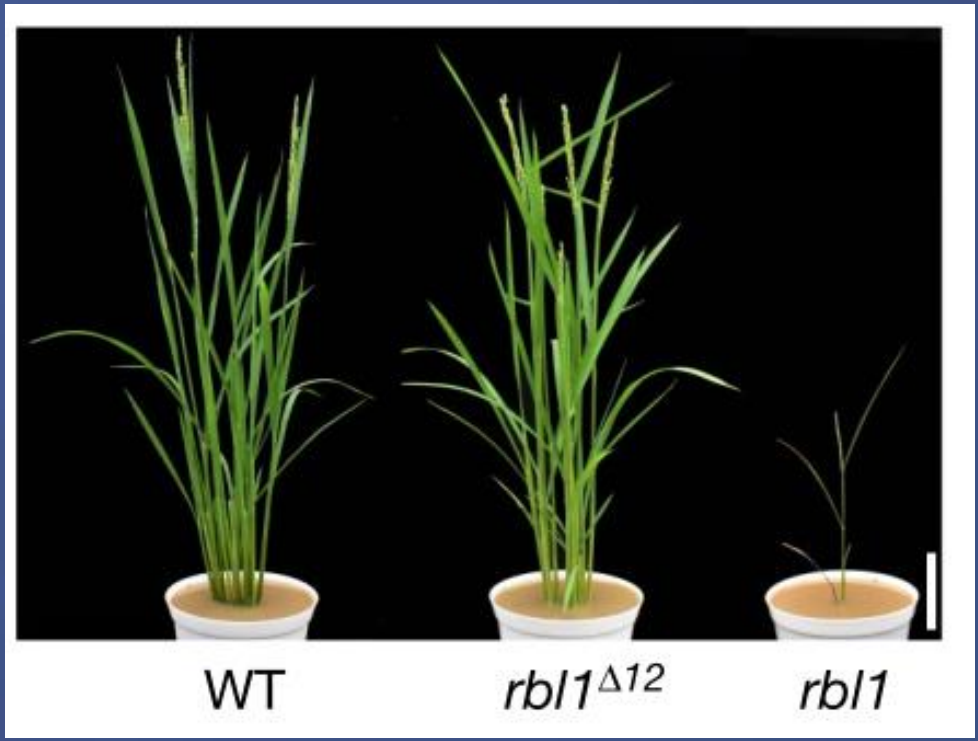
Sha, Gan, et al. "Genome editing of a rice CDP-DAG synthase confers multipathogen resistance." *Nature* (2023): 1-7.

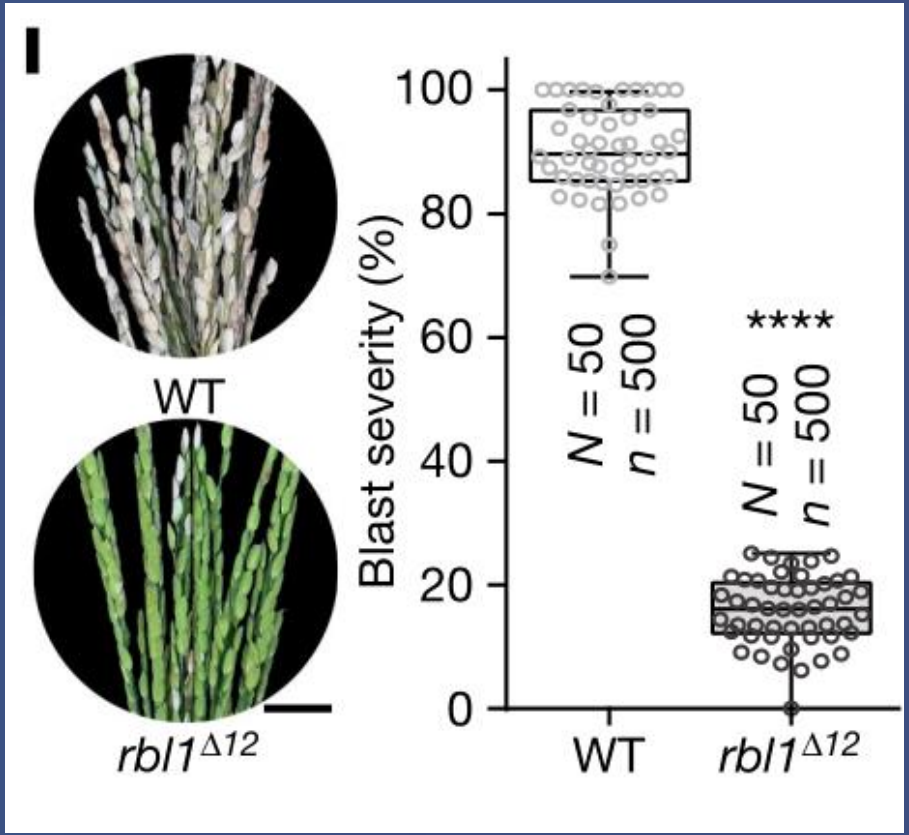


Virulence necessary host factor identified, HOWEVER, the plants were stunted

Target Genes and Modified Organisms

Sha, Gan, et al. "Genome editing of a rice CDP-DAG synthase confers multipathogen resistance." *Nature* (2023): 1-7.

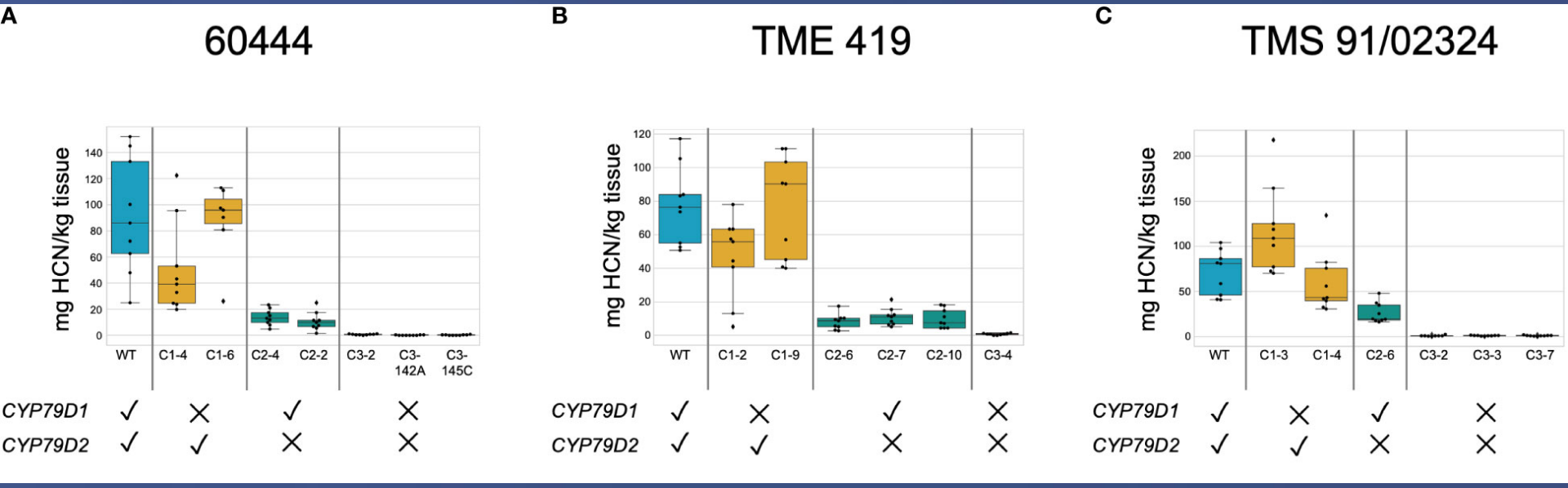




CRISPR enabled a “separation of function” resistance trait that otherwise had a yield loss

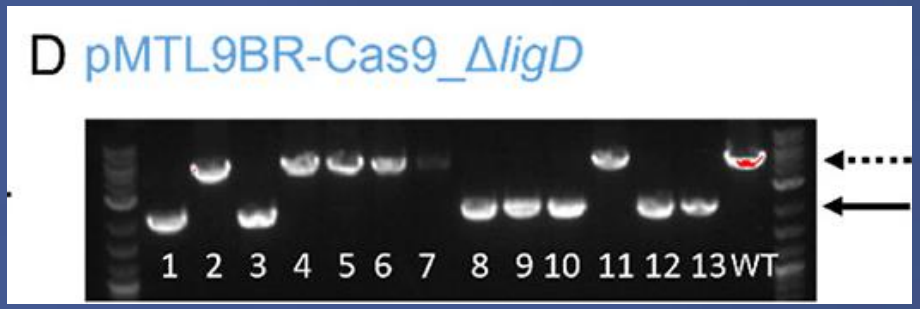
Target Genes and Modified Organisms

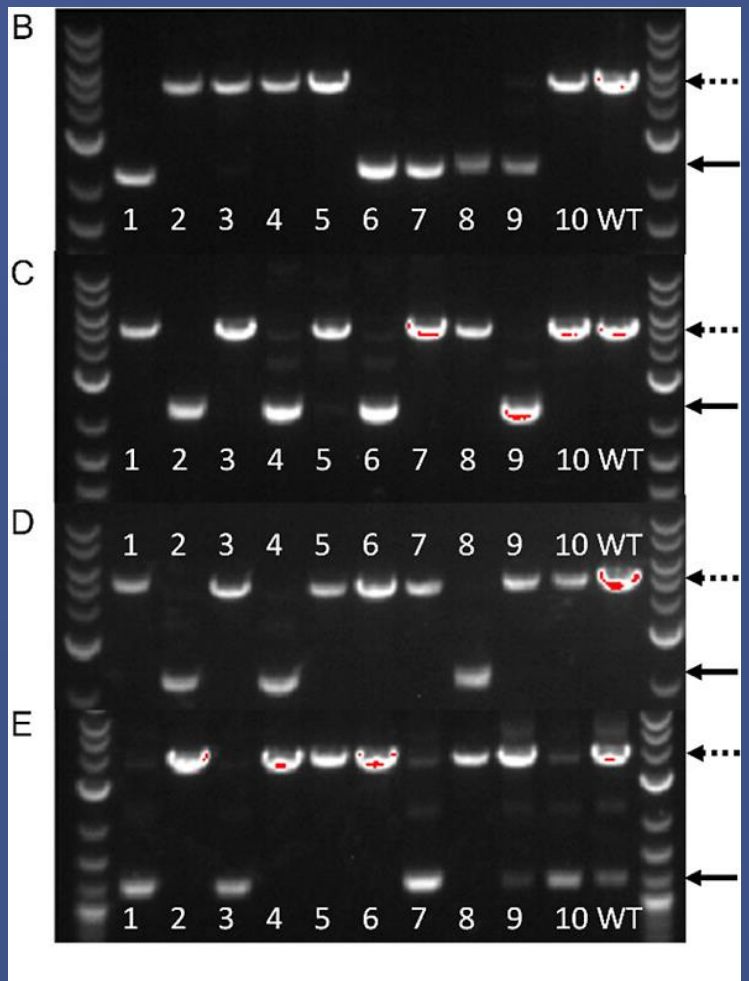
Gomez, Michael A., et al. "CRISPR-Cas9-mediated knockout of CYP79D1 and CYP79D2 in cassava attenuates toxic cyanogen production." *Frontiers in Plant Science* 13 (2023): 1079254.



Crop genome targets remain high value and high impact for drought resistance, disease resistance, pest resistance, and increased yield. In this case, a staple crop is also poisonous

Target Genes and Modified Organisms





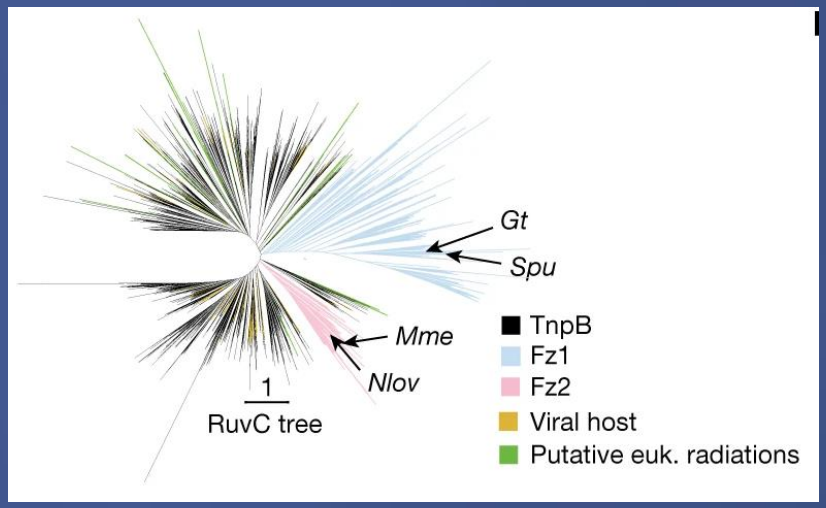
Rumah, Bashir L., et al. "In Vivo Genome Editing in Type I and II Methanotrophs Using a CRISPR/Cas9 System." *ACS Synthetic Biology* 12.2 (2023): 544-554.

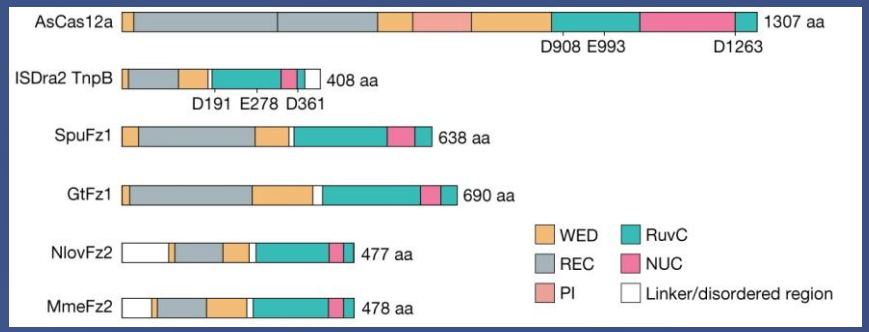
Methanotrophic bacteria are Gram-negative, aerobic organisms that use methane as their sole source of carbon and energy.

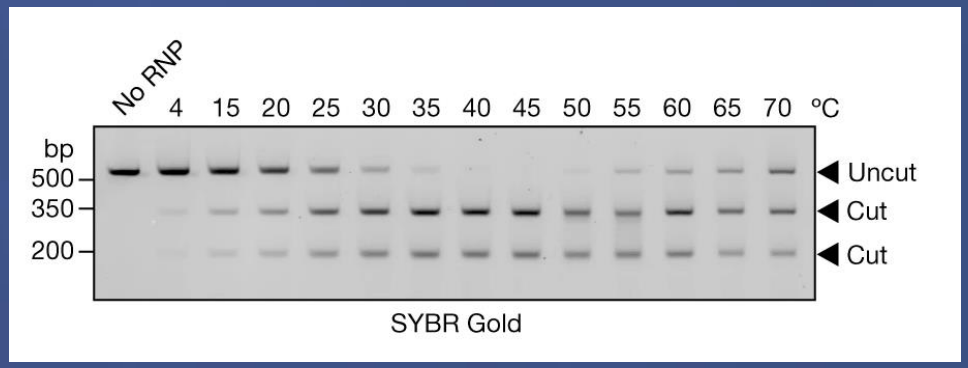
A way to convert inorganic carbon into organic and thus useful for combating carbon buildup in the atmosphere

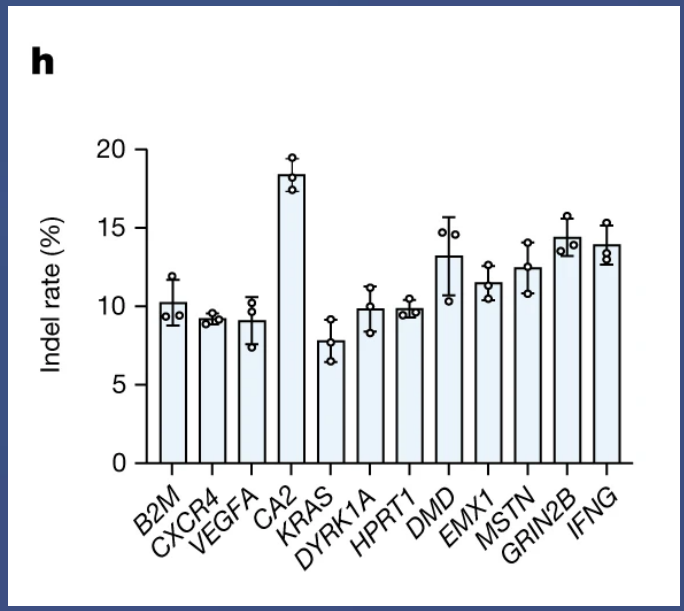
New Tools

Saito, Makoto, et al. "Fanzor is a eukaryotic programmable RNA-guided endonuclease." *Nature* 620.7974 (2023): 660-668.





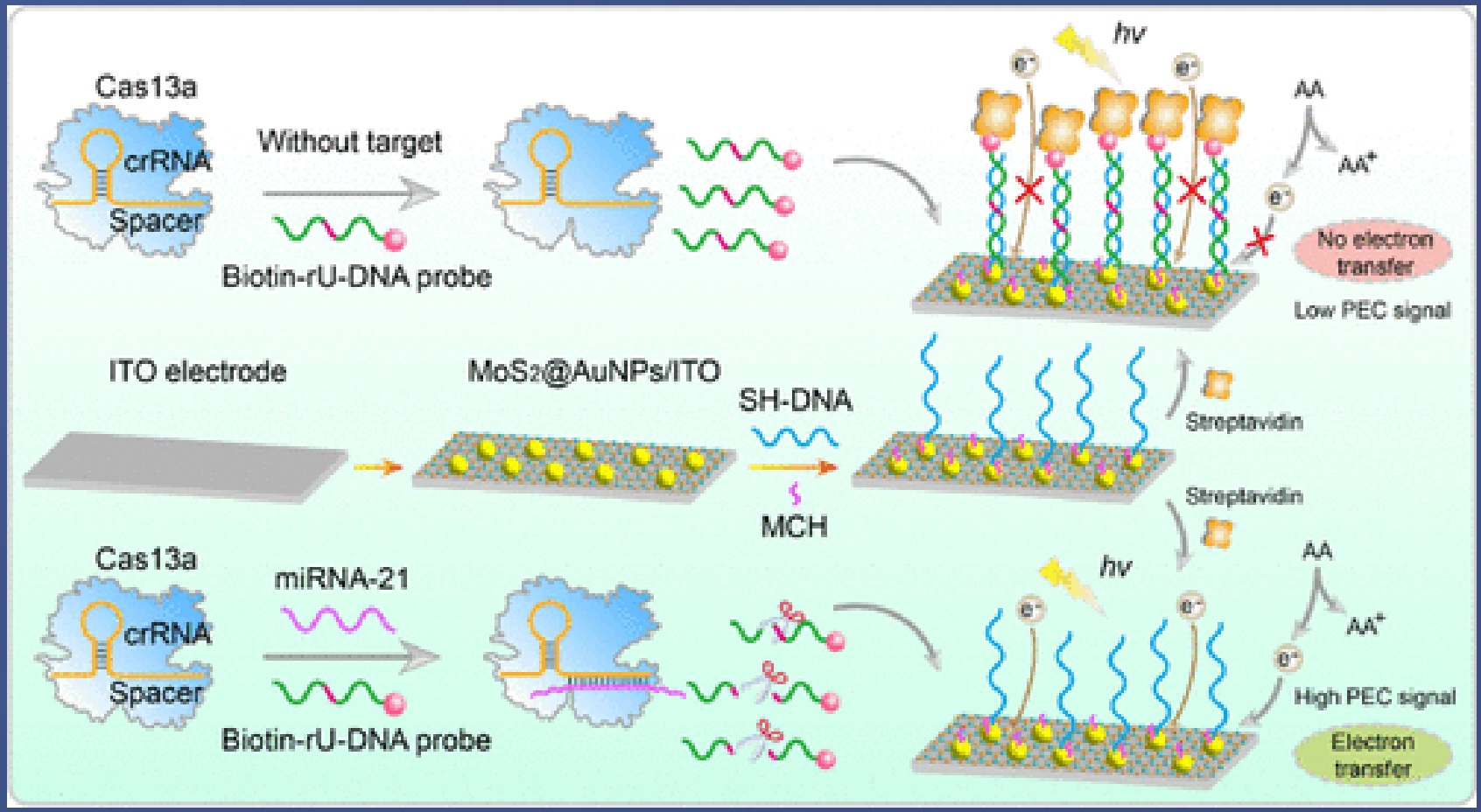




More the demonstration that CRISPR has evolved in eukaryotes and can be programmed. Unclear if any of these are better.

New Tools

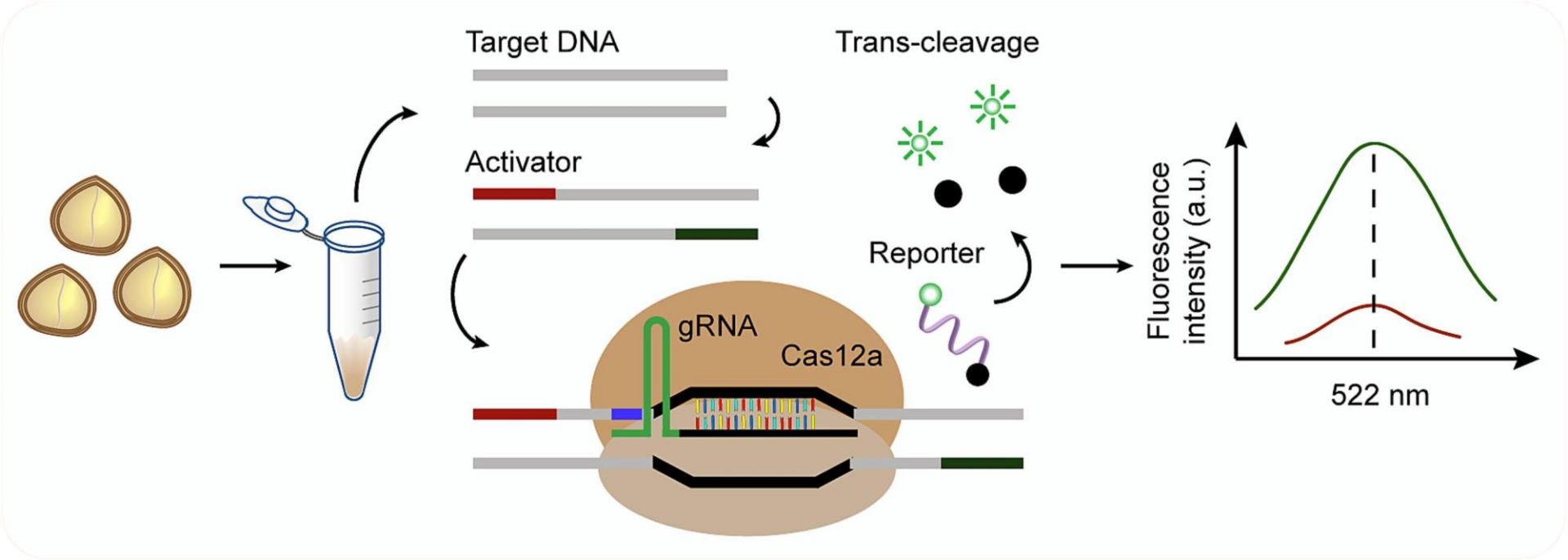
Jiang, Ling, et al. "Ultrasensitive CRISPR/Cas13a-Mediated photoelectrochemical biosensors for specific and direct assay of miRNA-21." *Analytical Chemistry* 95.2 (2023): 1193-1200.

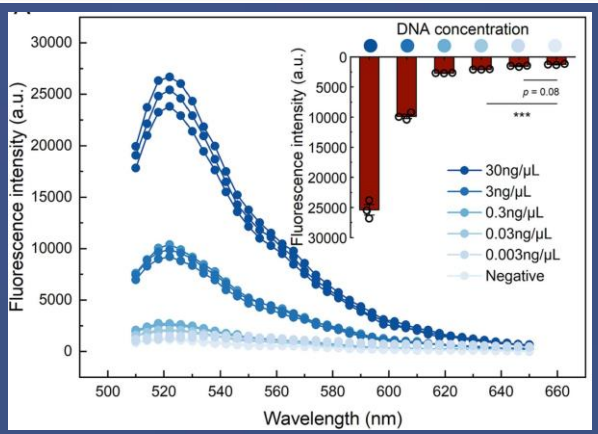


Novelty here is DIRECT detection of RNA with photoelectric biosensors without the RNA -> DNA intermediate

New Tools

Yin, Xinying, et al. "Development and validation of sensitive and rapid CRISPR/Cas12-based PCR method to detect hazelnut in unlabeled products." Food Chemistry 438 (2024): 137952.



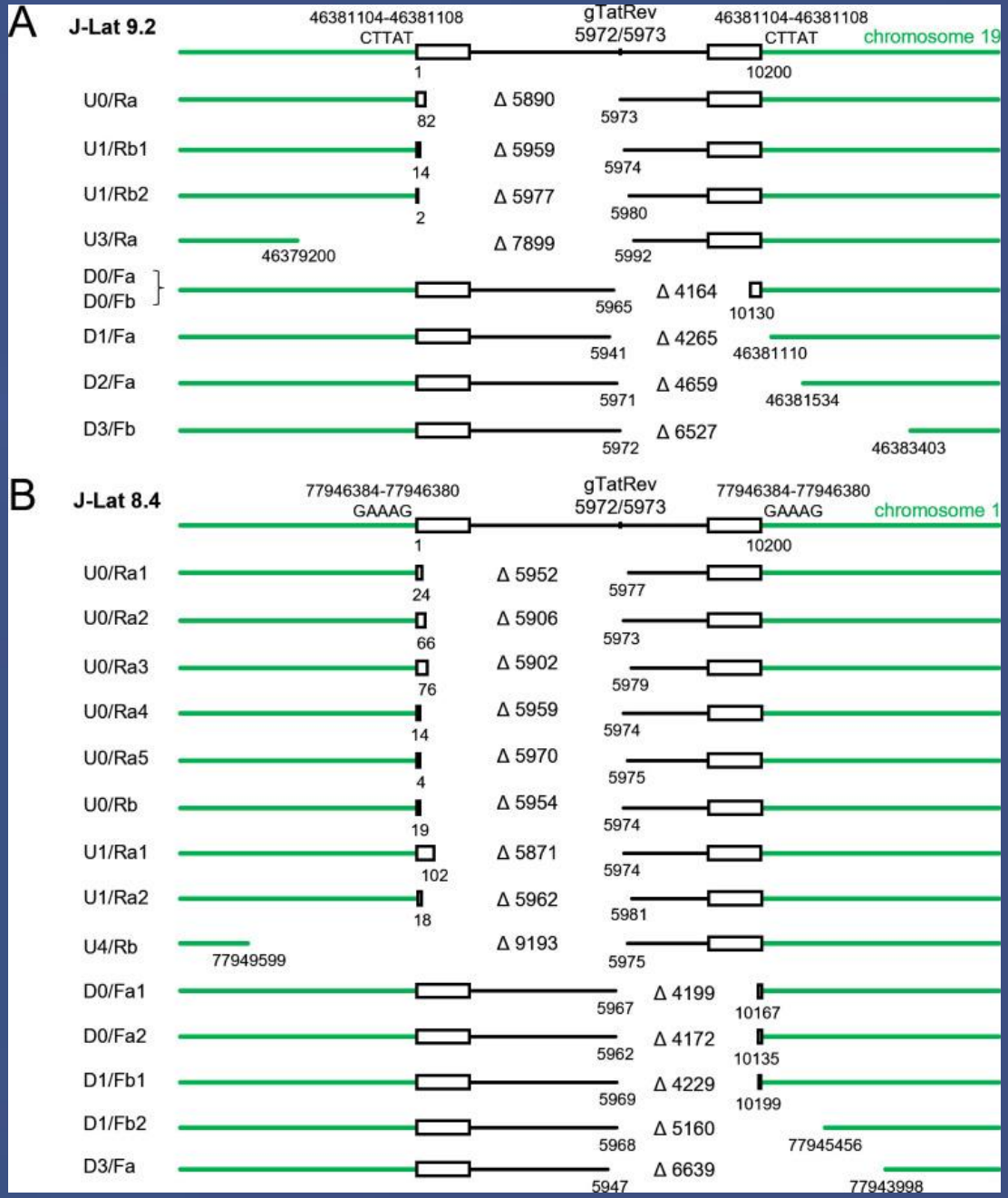


Developed to consumer use level. Don't need a thermocycler or other lab equipment

Cautionary Tales

Liu, Ye, et al. "CRISPR-Cas attack of HIV-1 proviral DNA can cause unintended deletion of surrounding cellular DNA." *Journal of Virology* (2023): e01334-23.

Deletions into green bars flanking the virus itself happen. NOT due to breaks being made in the flanking space.



Cautionary Tales

compared to conventional Cas nuclease-based editing, the CBE, ABE and PE decrease, but do not abolish, the occurrence of DNA DSBs at their genomic targeted sites, exposing cells to the potential genotoxic effects of deletions and translocations.

induced detrimental transcriptional responses in the treated cells that negatively impacted editing efficiency (for CBE) and/or clonogenic and repopulation capacity (for PE), albeit to a lesser extent than conventional nuclease-based editing.

Activation of IFN responses was observed after delivery of long and complex mRNA structures and may contribute to lower engraftment of edited cells, in particular for long-term repopulating progenitors.

Fiumara, Martina, et al. "Genotoxic effects of base and prime editing in human hematopoietic stem cells." ***Nature biotechnology*** **(2023): 1-15.**

Almost like Science Fiction

Evolutionary, High, et al. “Genetic engineering and cybernetic enhancement of *Procyon lotor* for bipedal locomotion, language, tool manipulation, and advanced cognitive function.” *Counter Nature* (2023): 1-15.

2023 CRISPR Year in Review

A very exciting year demonstrating

--Approved clinical therapy (awaiting insurance coverage)

--CRISPR food in US

--continued variety of specialized plant and animal applications

--continued variety of tools outside lab