# 2024 Genome Editing Year in Review

Science Circle
January 4th 2025

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# 2024 Genome Engineering Year in Review

Background

Therapies/To the Consumer Advances

Delivery Advances

Target Genes and Modified Organisms

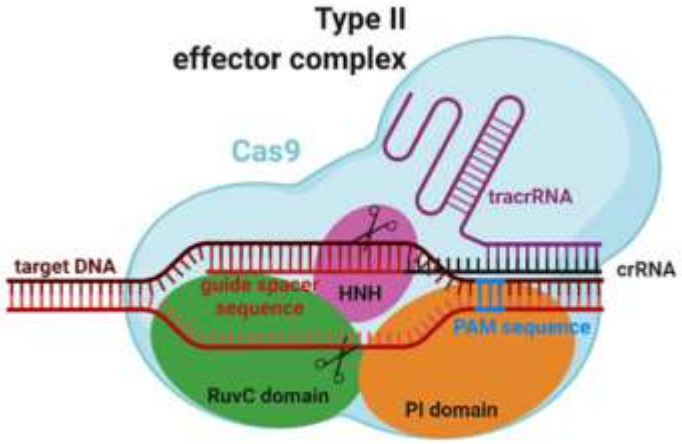
New Tools

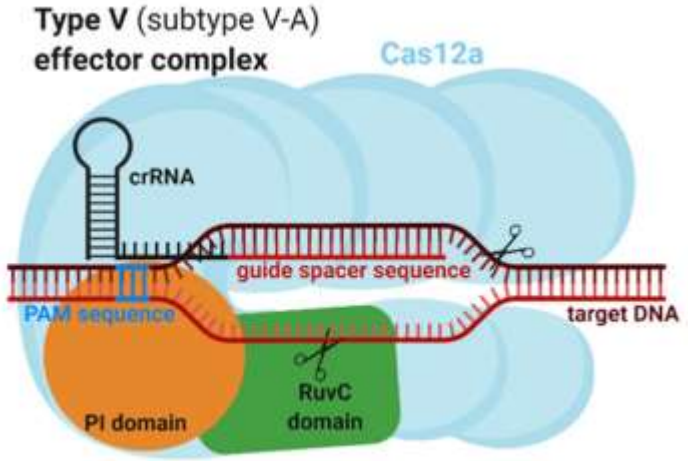
Cautionary Tales

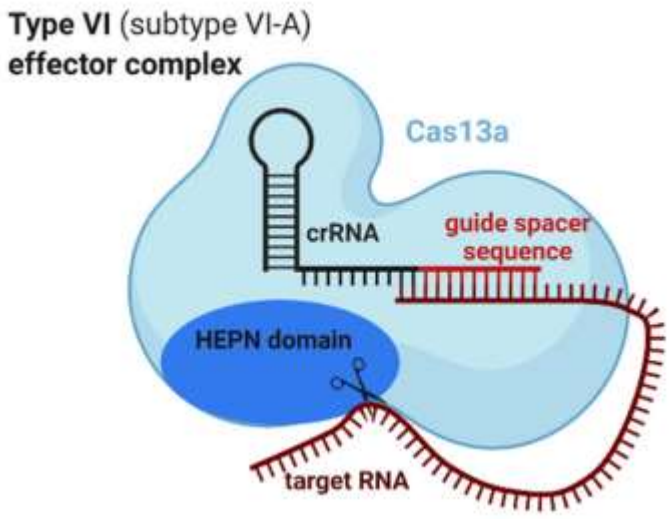
Researcher at Corteva. AgBioTech that operates in this space. Not representing the company's positions.

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

# 2024 Genome Engineering Year in Review




**Cas9 First and most studied and used**

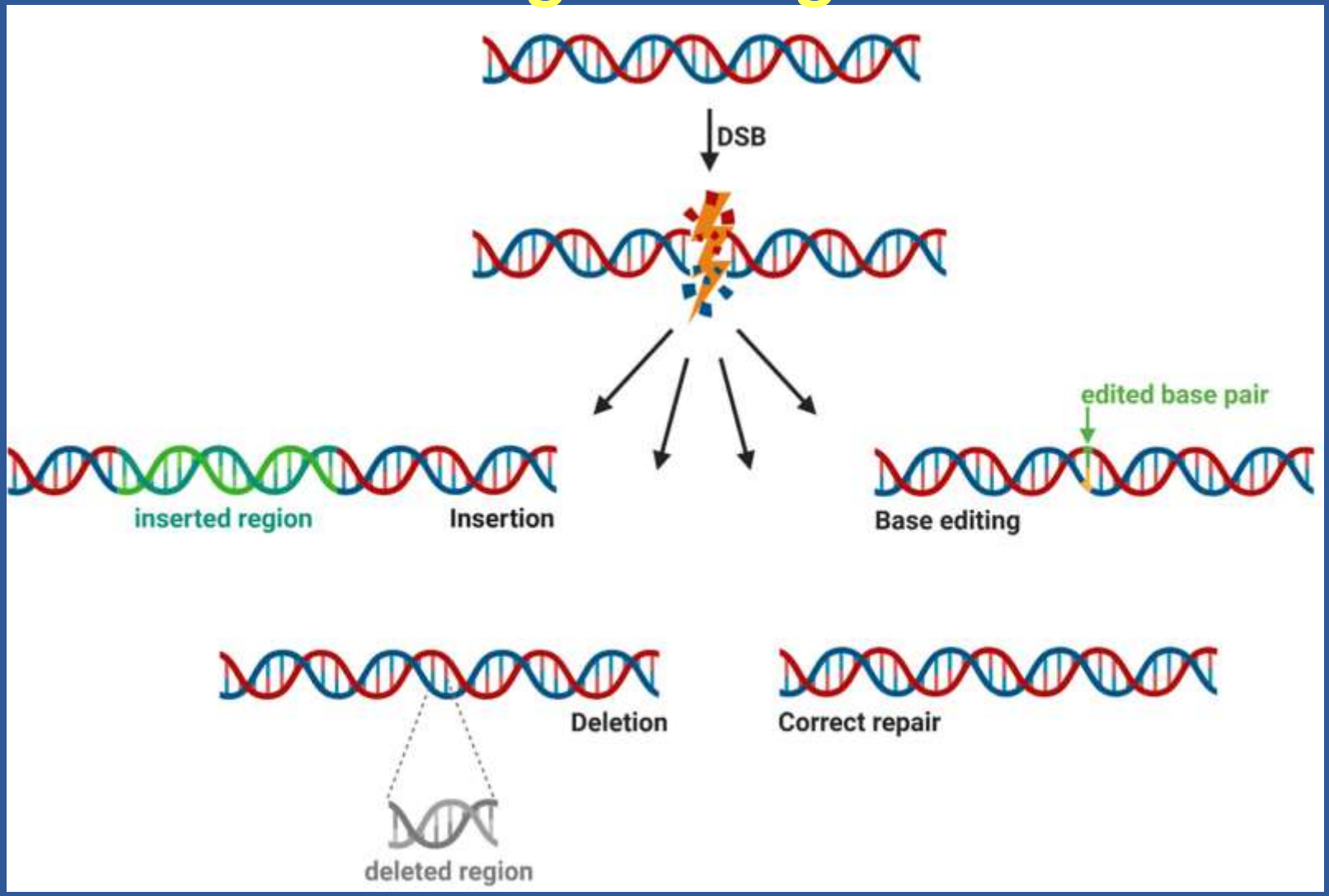
**Cas12a Second most well studied (large families of Cas12s)**

**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.

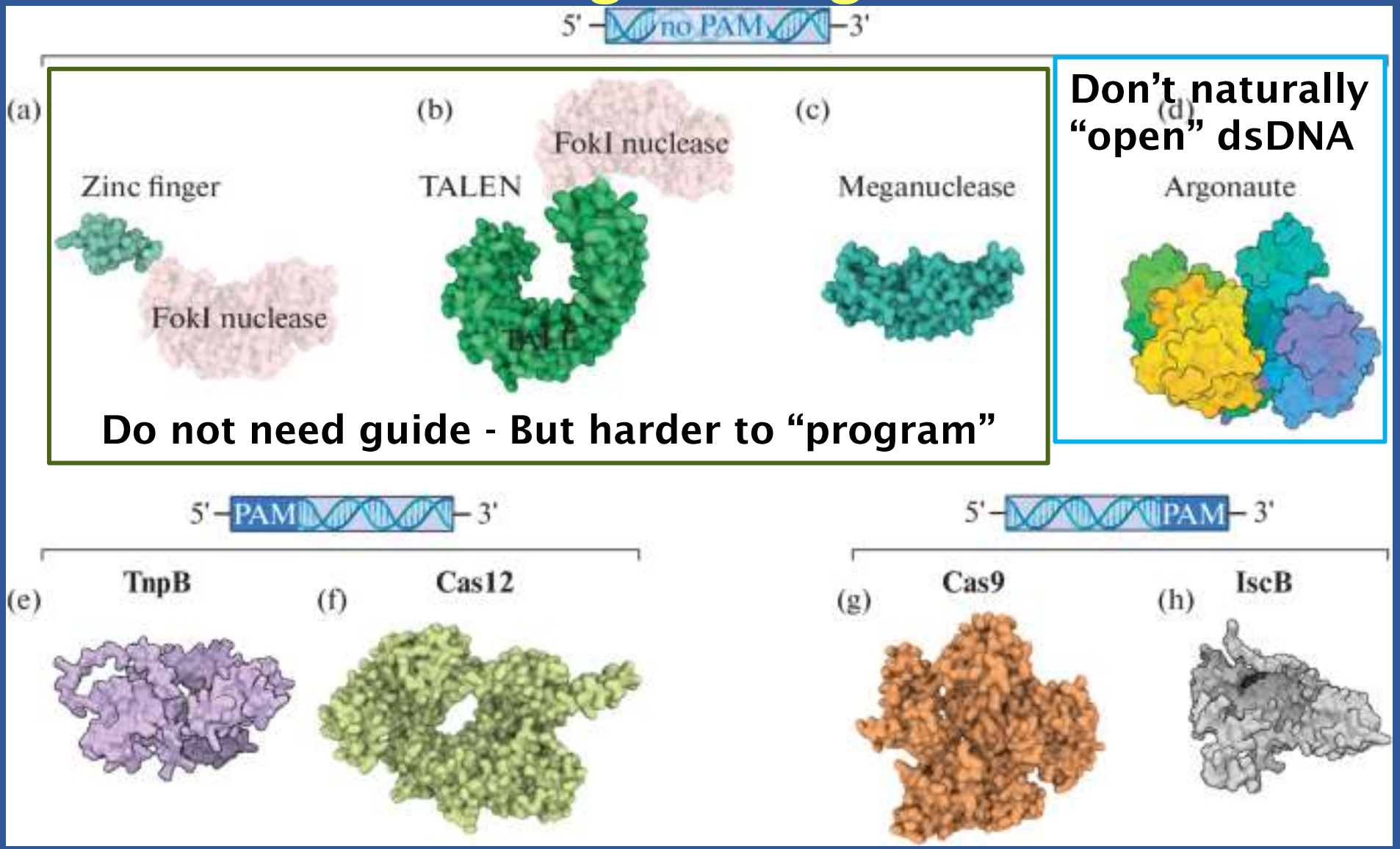
# 2024 Genome Engineering Year in Review




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.

# 2024 Genome Engineering Year in Review




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

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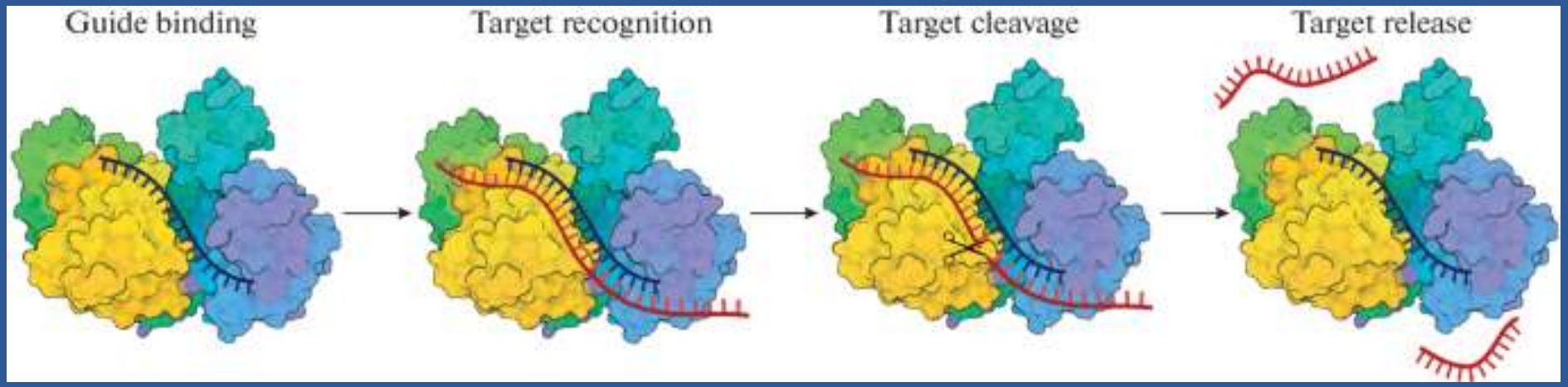


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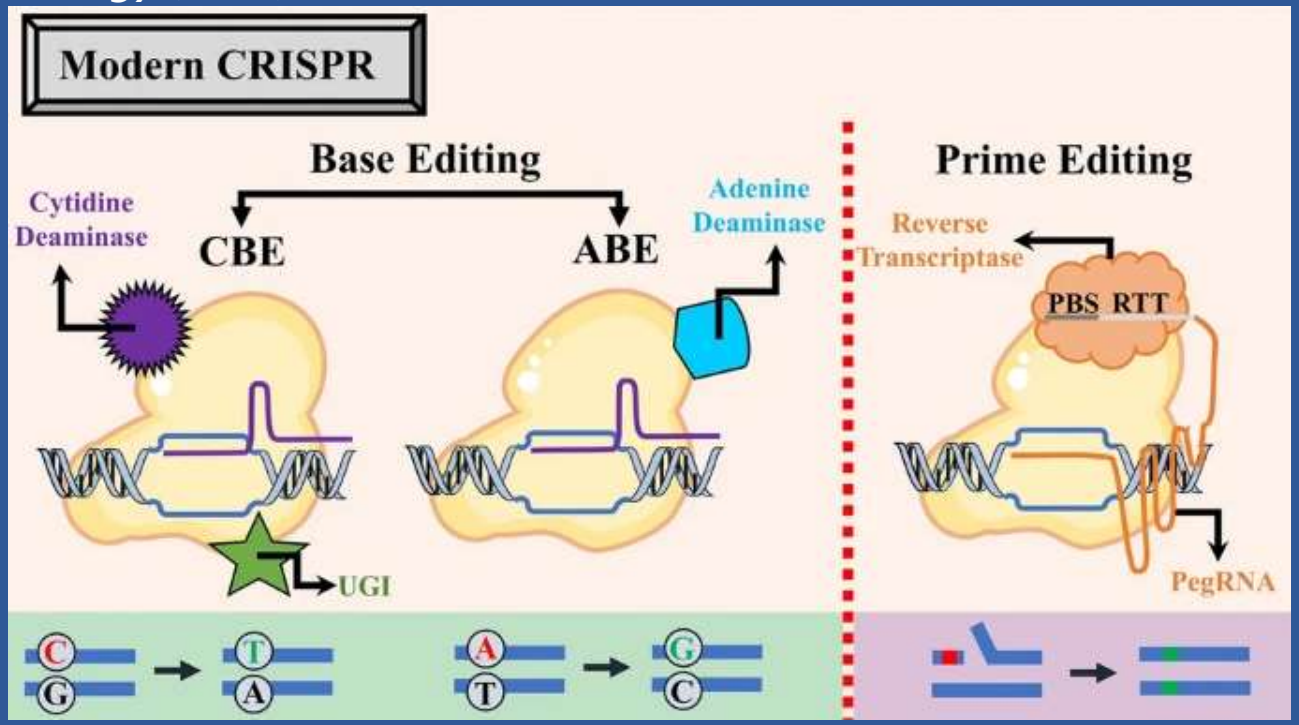



Saber Sichani, Ali, et al. "A Review on Advanced CRISPR-Based Genome-Editing Tools: Base Editing and Prime Editing." *Molecular Biotechnology* (2022): 1-12.

Insert novel DNA without a double-strand break

# Therapy Advances

## Intellia Announces First Clinical Evidence from Ongoing Phase 1 Study that Nexiguran Ziclumeran (nex-z), an In Vivo CRISPR/Cas9-Based Gene Editing Therapy, May Favorably Impact Disease Progression in Transthyretin (ATTR) Amyloidosis

https://ir.intelliatx.com/news-releases/news-release-details/intellia-announces-first-clinical-evidence-ongoing-phase-1-study

-Persistently deep levels of serum TTR reductions following a single infusion remain virtually unchanged after two or more years of follow-up in over 25 patients

-In newly reported data of multiple markers of (cardiac) disease progression, patients treated with nex-z showed evidence of disease stabilization or improvement at month 12 compared to baseline.

# Therapy Advances

**Pierce, Eric A., et al. "Gene editing for *CEP290*-associated retinal degeneration." *New England Journal of Medicine* 390.21 (2024): 1972-1984.**

-CEP290-associated inherited retinal degeneration causes severe early-onset vision loss due to pathogenic variants in CEP290. [cause dysfunction and death of the photoreceptor cells of the retina, resulting in visual impairment.]

-EDIT-101 was injected in 12 adults 17 to 63 years of age (median, 37 years) at a low dose (in 2 participants), an intermediate dose (in 5), or a high dose (in 5) and in 2 children 9 and 14 years of age at the intermediate dose. At baseline, the median best corrected visual acuity in the study eye was 2.4 log10 of the minimum angle of resolution (range, 3.9 to 0.6).

-EDIT-101 is a gene-editing therapy mediated by clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) that is designed to permanently remove the CEP290 IVS26 variant.

# Therapy Advances

**Longhurst, Hilary J., et al. "CRISPR-Cas9 in vivo gene editing of KLKB1 for hereditary angioedema." *New England Journal of Medicine* 390.5 (2024): 432-441.**

-Hereditary angioedema is a rare genetic disease that leads to severe and unpredictable swelling attacks. NTLA-2002 is an in vivo gene-editing therapy based on clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9. NTLA-2002 targets the gene encoding kallikrein B1 (KLKB1), with the goal of lifelong control of angioedema attacks after a single dose.

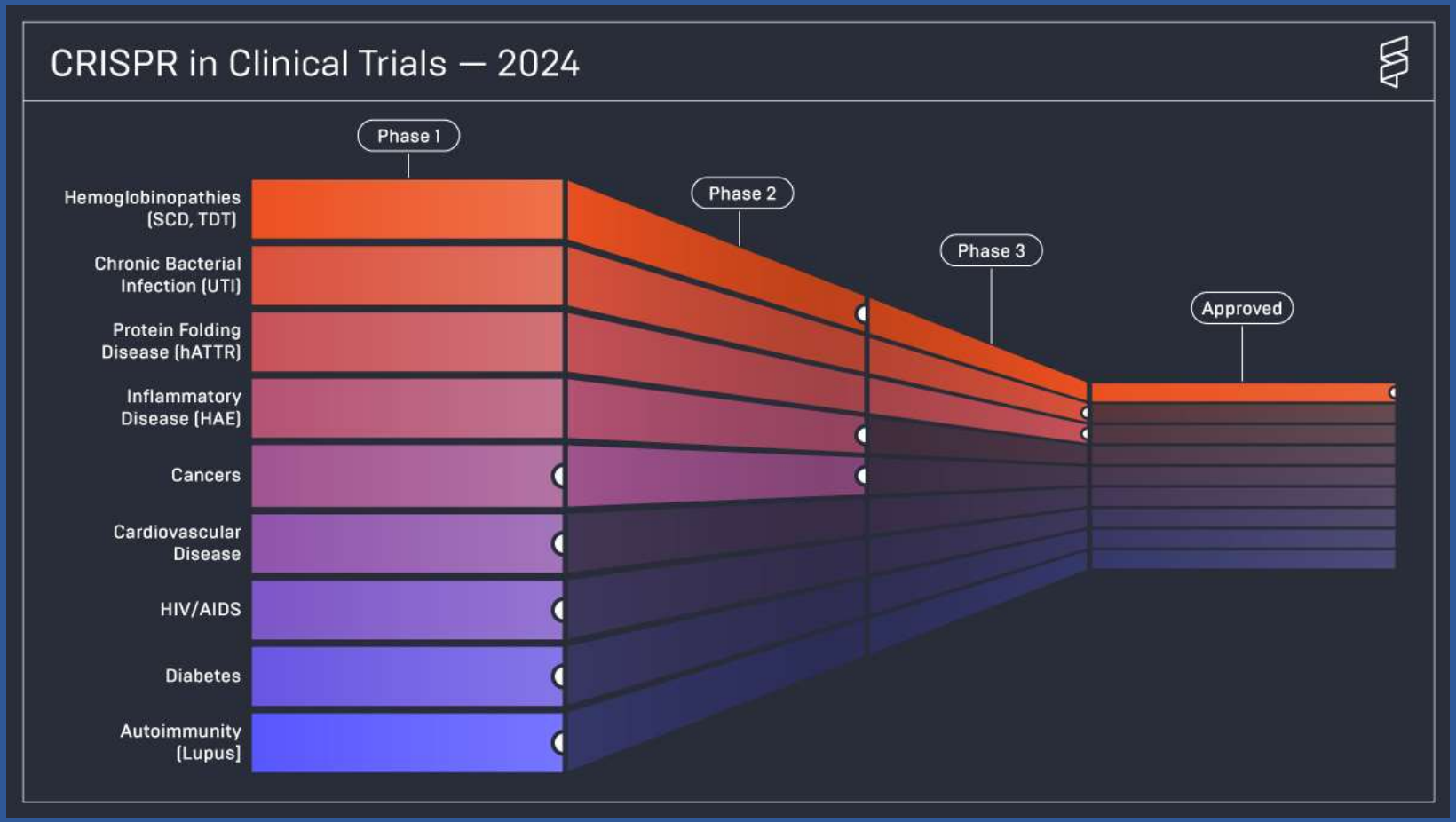
-No dose-limiting toxic effects, serious adverse events, grade 3 or higher adverse events, or clinically important laboratory findings were observed after the administration of NTLA-2002.

-Dose-dependent reductions in the total plasma kallikrein protein level were observed between baseline and the latest assessment, with a mean percentage change of −67% in the 25-mg group, −84% in the 50-mg group, and −95% in the 75-mg group. The mean percentage change in the number of angioedema attacks per month between baseline and weeks 1 through 16 (primary observation period) was −91% in the 25-mg group, −97% in the 50-mg group, and −80% in the 75-mg group.

# Therapy Advances

CRISPR Clinical Trials: A 2024 Update
March 13, 2024 Perspectives
By Hope Henderson

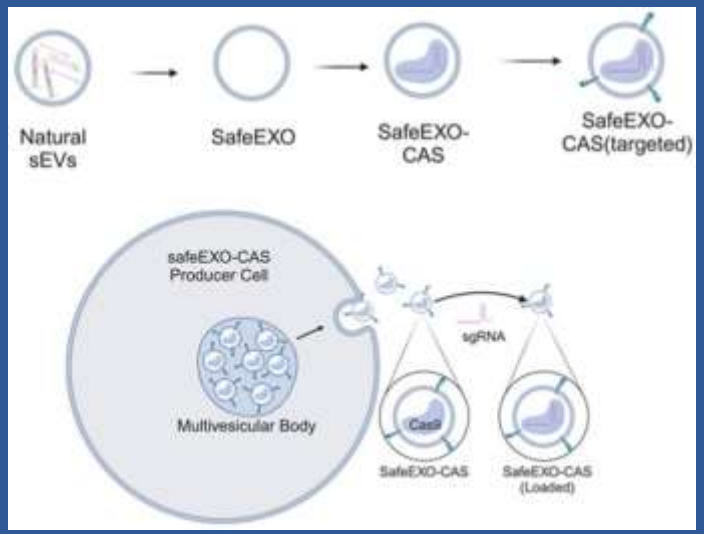
Innovative Genomics Institute



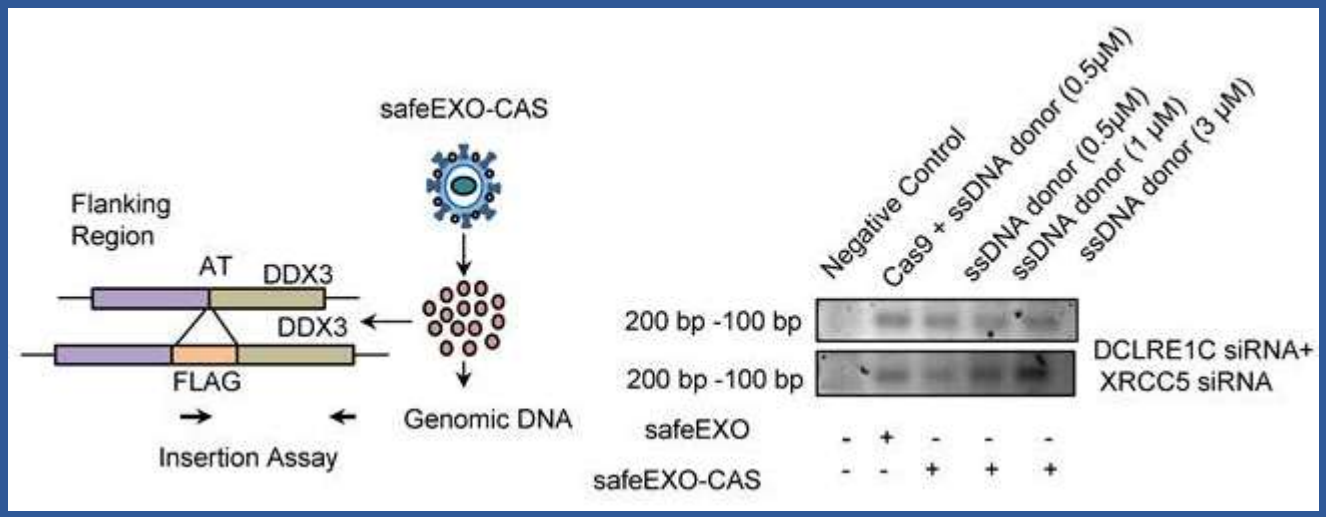

https://innovativegenomics.org/news/crispr-clinical-trials-2024/

# Delivery Advances

Dubey, Sunil, et al. "Small extracellular vesicles (sEVs)-based gene delivery platform for cell-specific CRISPR/Cas9 genome editing." *Theranostics* 14.7 (2024): 2777.




The results presented in this study demonstrate the potential of the safeEXO platform as a novel, non-invasive method to deliver RNAi and CRISPR-based gene editing components for high-efficiency delivery and genomic editing. We showed that safeEXO-CAS sEVs can effectively deliver highly complex payloads, including sgRNA, ssDNA, and siRNAs, a novel strategy that we demonstrate can block NHEJ and facilitate HDR-based gene insertions [also tissue specificity]

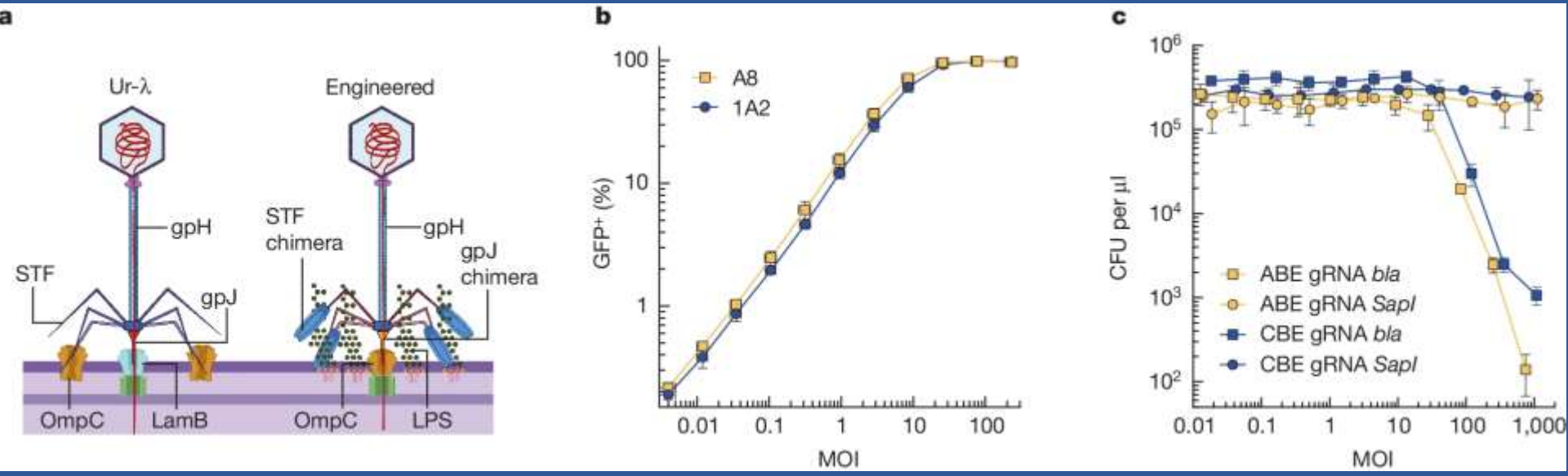



Multiple and complex payloads is an advance over other nano-particles and viral vectors

# Delivery Advances

Brödel, Andreas K., et al. "In situ targeted base editing of bacteria in the mouse gut." *Nature* 632.8026 (2024): 877-884.

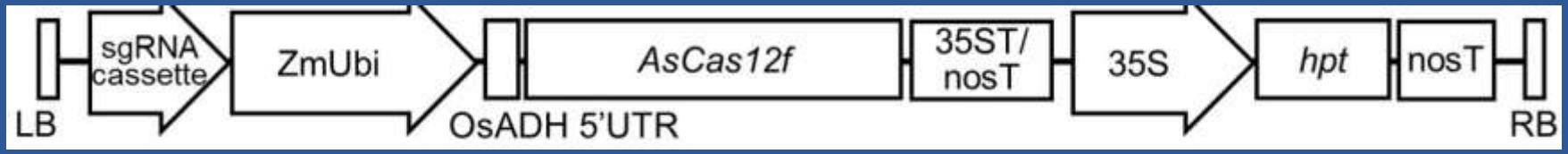
We demonstrate the efficient and durable genetic modification of a bacterial population in the gut environment using engineered, non-replicative, phage-derived, delivery particles equipped with base editors. We engineered the λ tail to ensure binding to surface determinants consistently expressed by *E. coli* in the gut environment, thereby enabling delivery to most of a target population without resorting to selection.



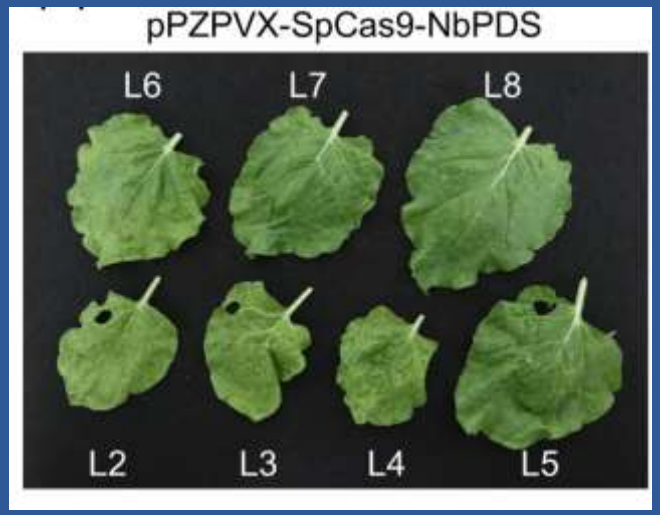

Microbiome engineering can be useful for long-term health in addition to being a way to combat pathogenic

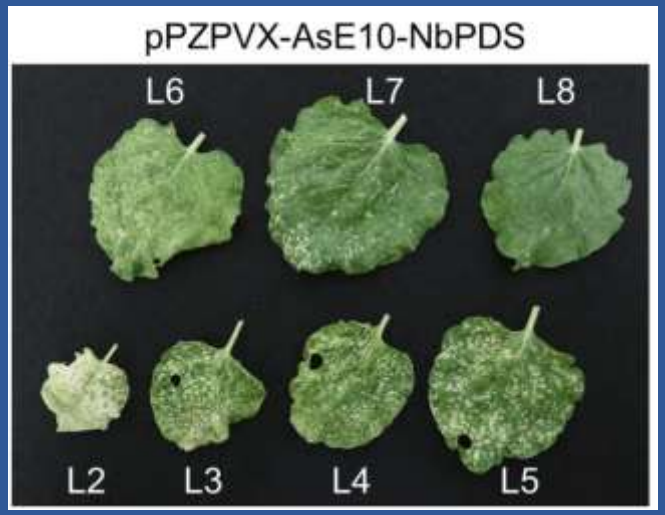
# Delivery Advances

Ishibashi, Kazuhiro, et al. "Systemic delivery of engineered compact AsCas12f by a positive-strand RNA virus vector enables highly efficient targeted mutagenesis in plants." *Frontiers in Plant Science* 15 (2024): 1454554.




"Taken together, our results demonstrate that AsCas12f is small enough to be maintained in the PVX [RNA viral derived] vector during systemic infection in *N. benthamiana* and that engineered AsCas12f offers advantages over SpCas9 for plant genome editing using virus vectors."



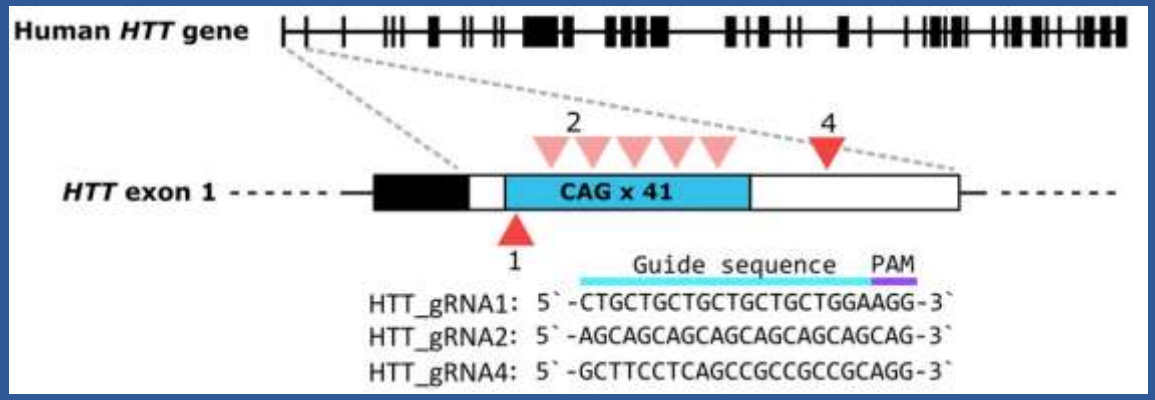



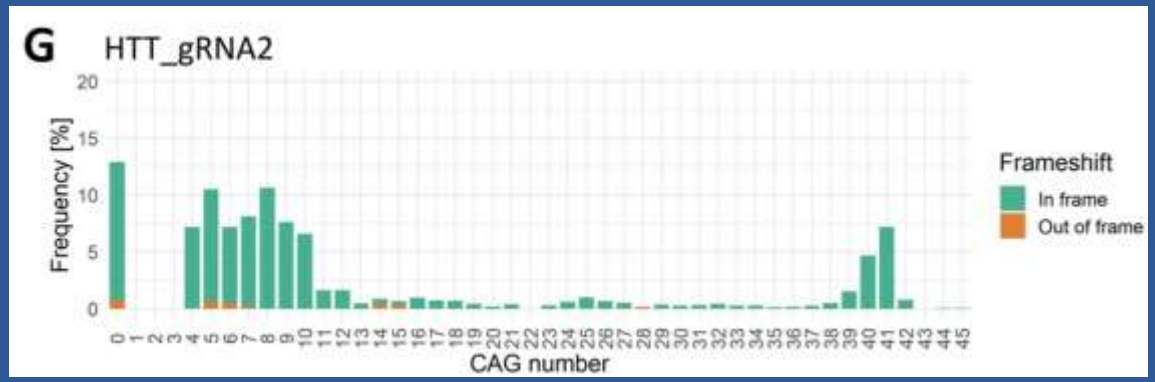

Alternatives to Cas9 are improving and better in at least some specialized cases

# Target Genes and Modified Organisms

Sledzinski, Pawel, et al. "CRISPR/Cas9-induced double-strand breaks in the huntingtin locus lead to CAG repeat contraction through DNA end resection and homology-mediated repair." *BMC biology* 22.1 (2024): 1-21.

"Expanded CNG trinucleotide repeats have been found to contribute to neurodegenerative disorders, e.g., Huntington's disease (HD),…we try to identify the key mechanisms responsible for the shortening of the CAG tract by studying the phenomena that occur after SpCas9-mediated induction of DSBs in the CAG microsatellite region of the HTT gene."

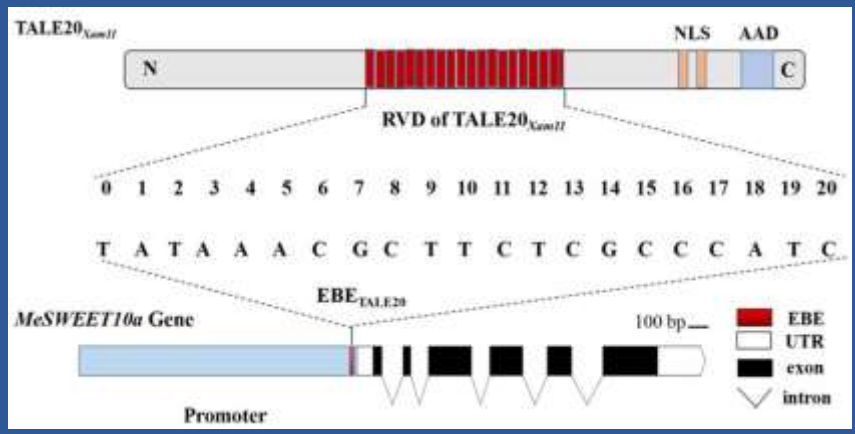


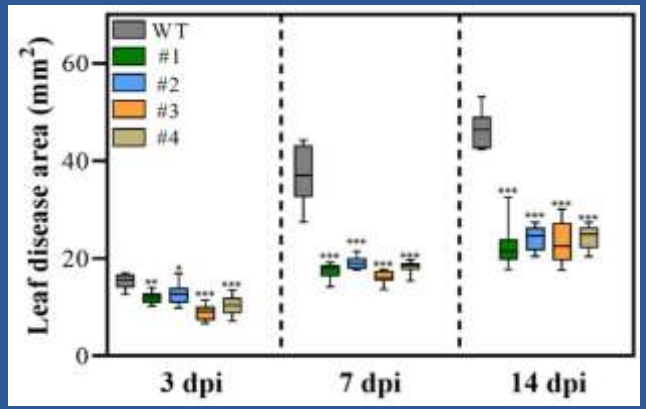

Using CRISPR to understand DNA repair changes but also a potential way to reverse the disease mutation

# Target Genes and Modified Organisms

**Wang, Yajie, et al. "Editing of the *MeSWEET10a* promoter yields bacterial blight resistance in cassava cultivar SC8." *Molecular Plant Pathology* 25.10 (2024): e70010.**




## Important but regionally valued crops amenable to modification


Cassava is an important crop for tropical and subtropical regions. The tuberous roots of cassava are rich in starch (20%–40%), which is an essential food source for 800 million people in the world (Prochnik et al., 2012). It has strong tolerance to drought and low-fertility environments and is extensively cultivated throughout the tropical and subtropical regions. However, the production of cassava is severely affected by biotic stresses, such as cassava brown streak disease (CBSD) [*Xanthomonas axonopodis pv. manihotis (Xam)* ], cassava bacterial blight (CBB), whiteflies, mealybugs and green mites.

# Target Genes and Modified Organisms

Agriculture plants for consumers or farmers rundown

Pairwise Develops First Seedless Blackberry with Transformative CRISPR Technology
Heather Barefoot
Jun 4, 2024 https://www.pairwise.com/news/pairwise-develops-first-seedless-blackberry

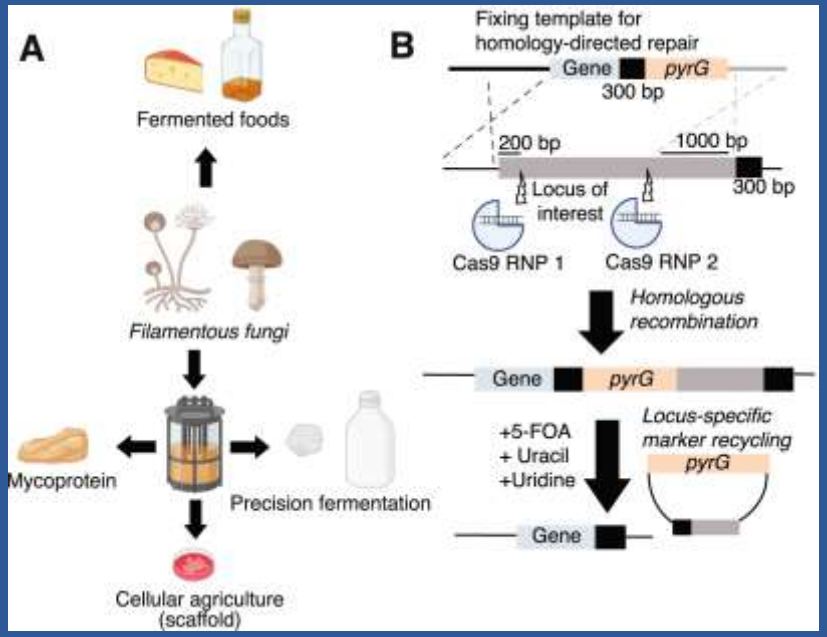
New non-browning bananas in the Philippines
July 03 , 2024
classified as non-genetically modified organisms
https://www.freshfruitportal.com/news/2024/07/03/new-non-browning-bananas-in-the-philippines/

Here's your chance to try CoverCress
The CoverCress Farm Adoption Program allows farmers to test out this new combination cash and cover crop.
Allison Lynch, Indiana Prairie Farmer Senior Editor
May 24, 2024
https://www.farmprogress.com/cover-crops/here-s-your-chance-to-try-covercress

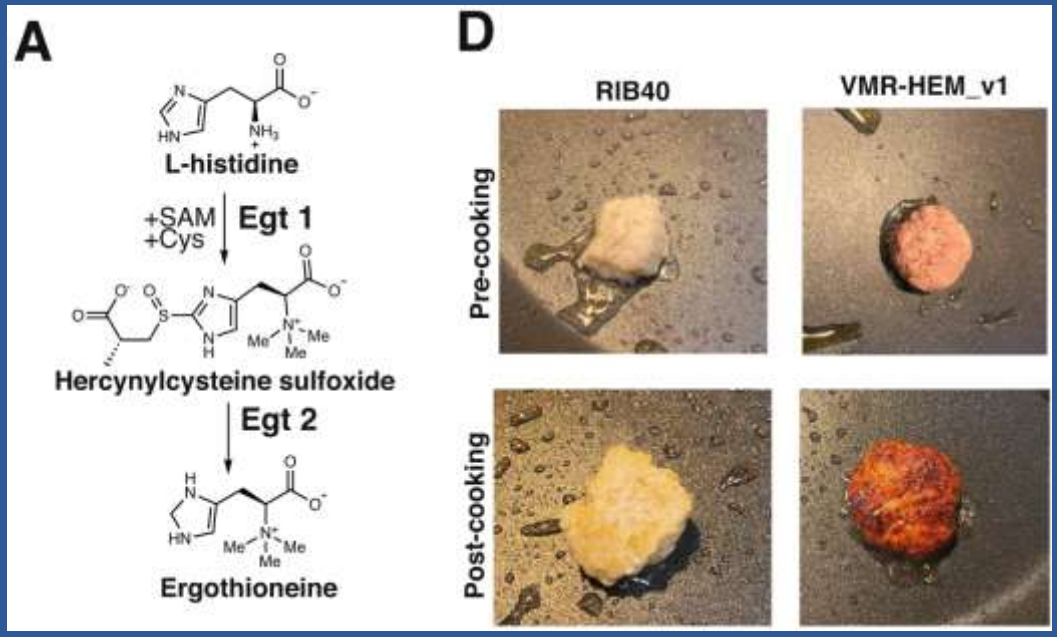
# Target Genes and Modified Organisms

Maini Rekdal, Vayu, et al. "Edible mycelium bioengineered for enhanced nutritional value and sensory appeal using a modular synthetic biology toolkit." *Nature Communications* 15.1 (2024): 2099.

we develop a modular synthetic biology toolkit for *Aspergillus oryzae*, an edible fungus used in fermented foods, protein production, and meat alternatives.




Molecular toolkit development

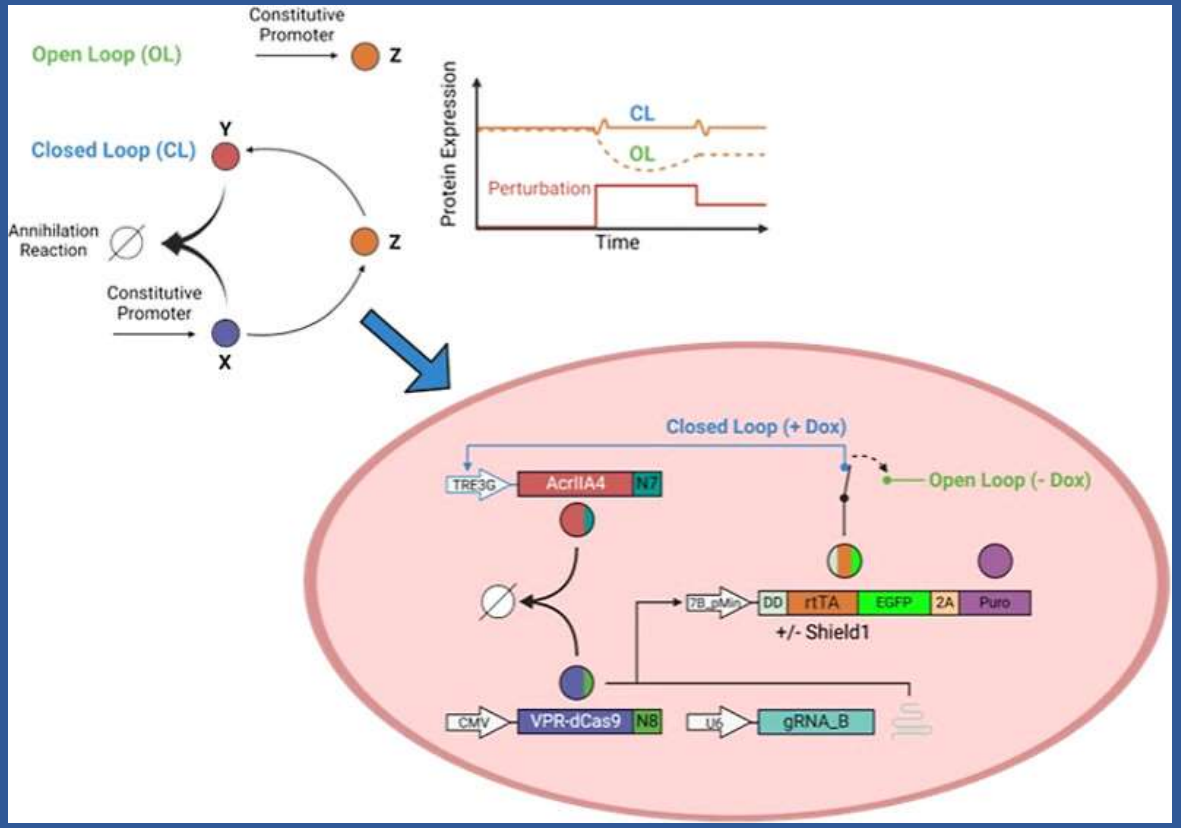



Increased biosynthesis of ergothioneine, a powerful antioxidant
And cooking of heme containing fungus

# New Tools

Mallozzi, Alessio, et al. "A Biomolecular Circuit for Automatic Gene Regulation in Mammalian Cells with CRISPR Technology." *ACS Synthetic Biology* (2024).

"maintain the expression of a reporter protein constant across diverse experimental conditions, including fluctuations in protein degradation rates and plasmid concentrations, by automatically adjusting its mRNA level. This capability, known as robust perfect adaptation (RPA)"

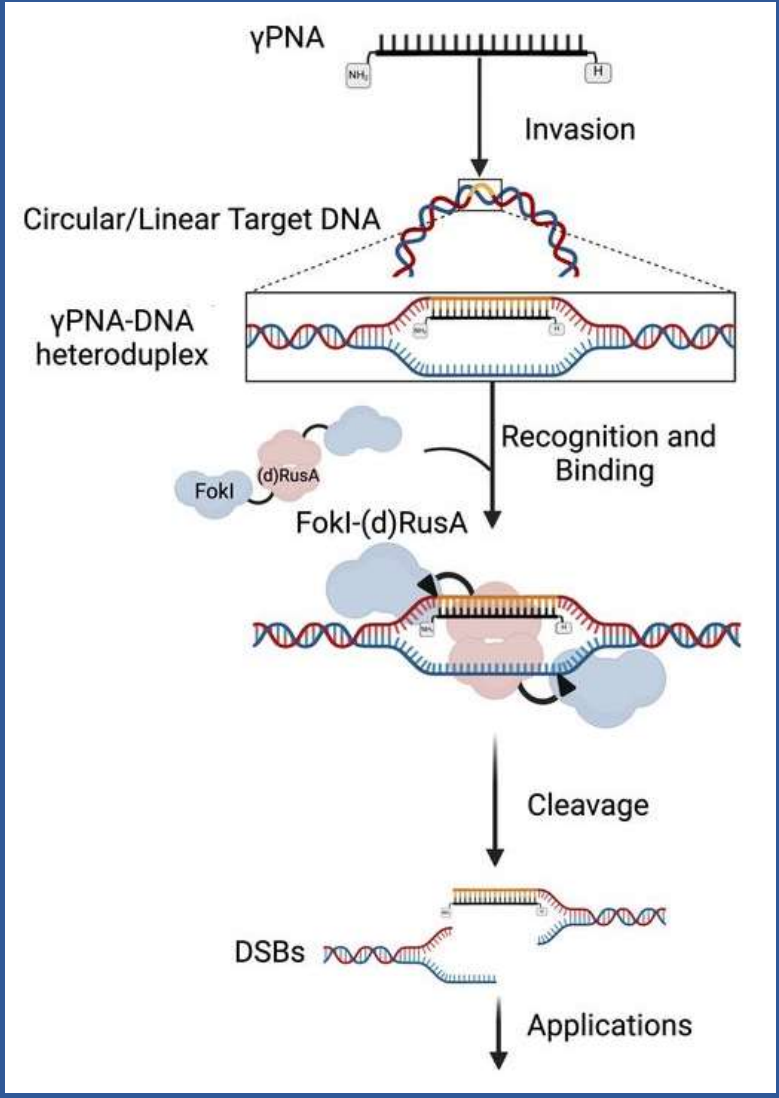



Useful technology for building engineered and embedded expression control

But also for inducible, suppressible, etc

# New Tools

Mahfouz, Magdy, et al. "Fusions of catalytically inactive RusA to FokI nuclease coupled with PNA enable programable site-specific double-stranded DNA breaks." *bioRxiv* (2024): 2024-12.




PC-FIRA system can induce DSBs with a single PNA invasion, therefore, it offers a more cost-efficient and less complex gene editing tool. It enhances specificity by utilizing PNAs to target complex and highly specific DNA structures, reducing off-target effects.

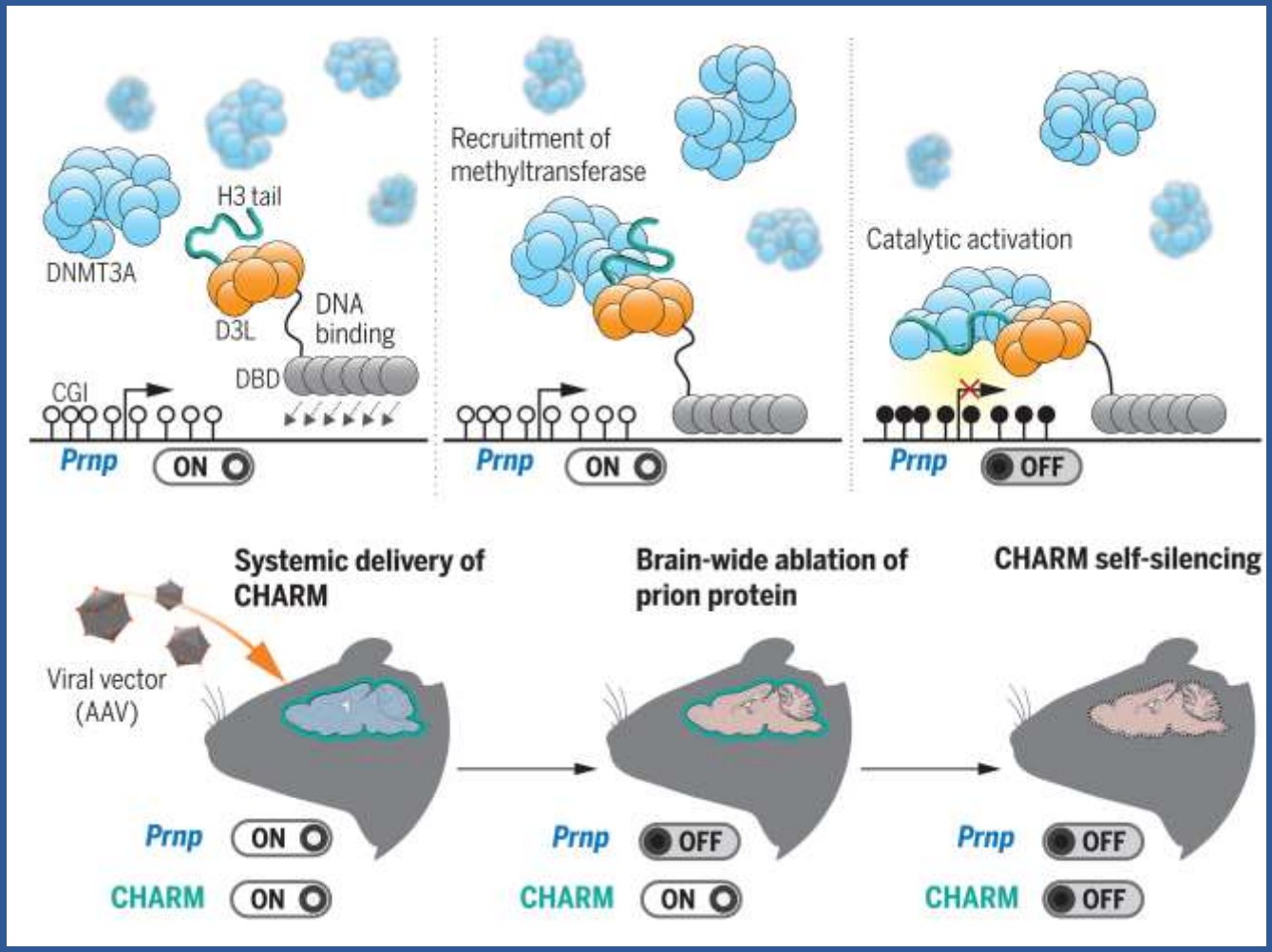
Several utilities to this system:

gRNA independent
Structure recognition so fewer off-target effects

FokI duality also limits off target activity

# New Tools

Neumann, Edwin N., et al. "Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor." *Science* 384.6703 (2024): ado7082.



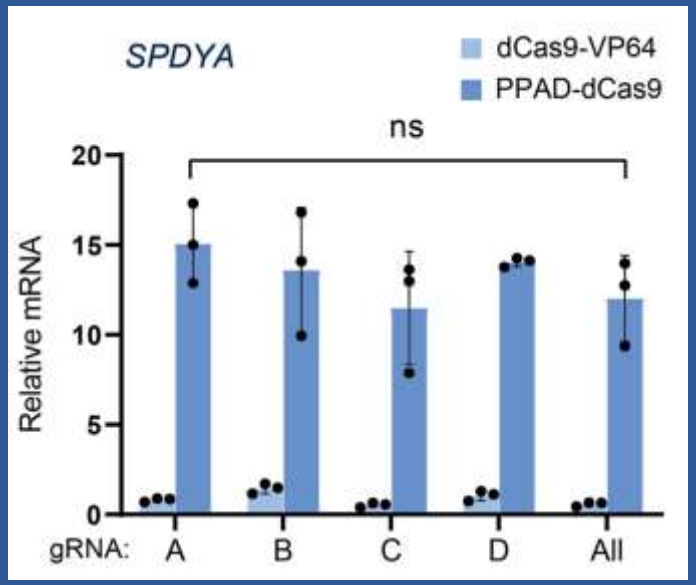

"enzyme-free epigenetic editor termed CHARM (Coupled Histone tail for Autoinhibition Release of Methyltransferase). Through a direct fusion with the histone H3 tail and a noncatalytic Dnmt3l domain, CHARM is able to recruit and activate DNA methyltransferases endogenously expressed in the cell to methylate the target gene."
"CHARM methylates the prion gene promoter and achieves up to 80% brainwide reduction in neuronal prion protein"

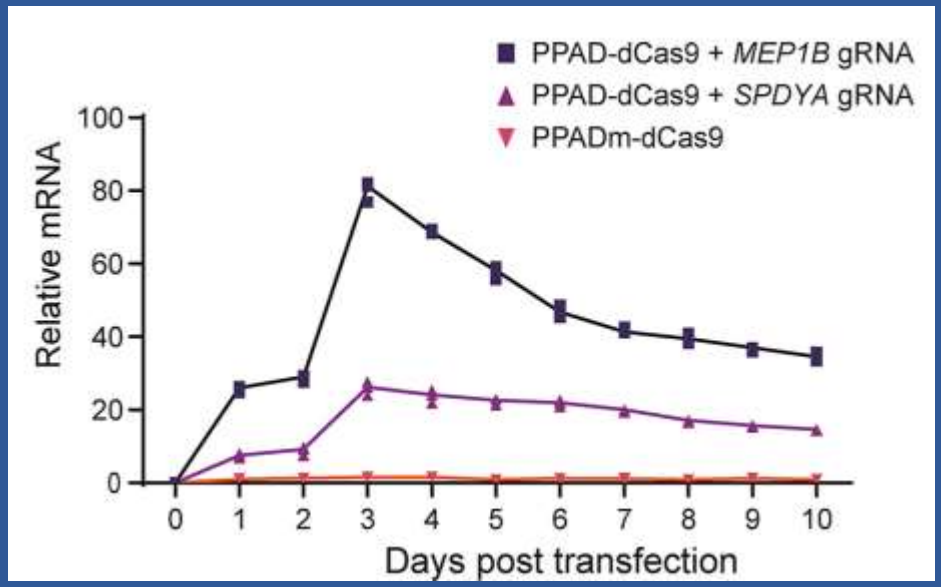
Targeted epigenetics for turning off genes – very compact system

# New Tools

Zhang, Xiaoya, et al. "A programmable CRISPR/dCas9-based epigenetic editing system enabling loci-targeted histone citrullination and precise transcription regulation." *Journal of Genetics and Genomics* (2024).

With the assistance of gRNA, PPAD-dCas9 can recruit PPADs [peptidyl arginine deiminase] to specific genomic loci, enabling direct manipulation of the epigenetic landscape and regulation of gene expression. Our citrullination editor allows for the site-specific manipulation of histone H3R2,8,17 and H3R26 at target human gene loci, resulting in the activation or suppression of different genes in a locus-specific manner.



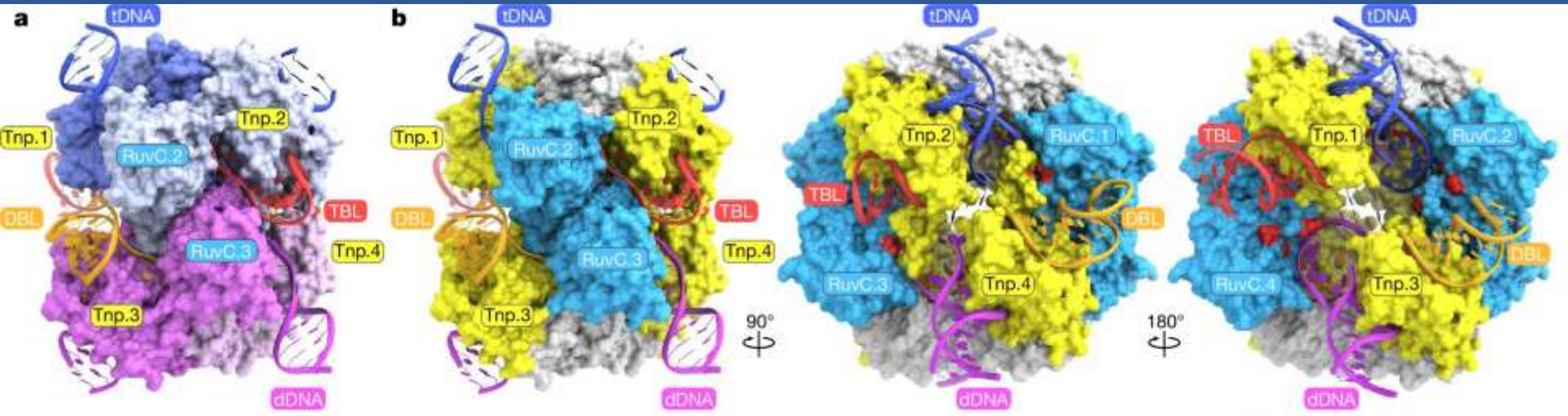




An additional histone target for expression control but same idea as ibid but in contrast can be gene activation

# New Tools

Hiraizumi, Masahiro, et al. "Structural mechanism of bridge RNA-guided recombination." *Nature* 630.8018 (2024): 994-1002.

Insertion sequence (IS) elements are the simplest autonomous transposable elements found in prokaryotic genomes1. We recently discovered that IS110 family elements encode a recombinase and a non-coding bridge RNA (bRNA) that confers modular specificity for target DNA and donor DNA through two programmable loops

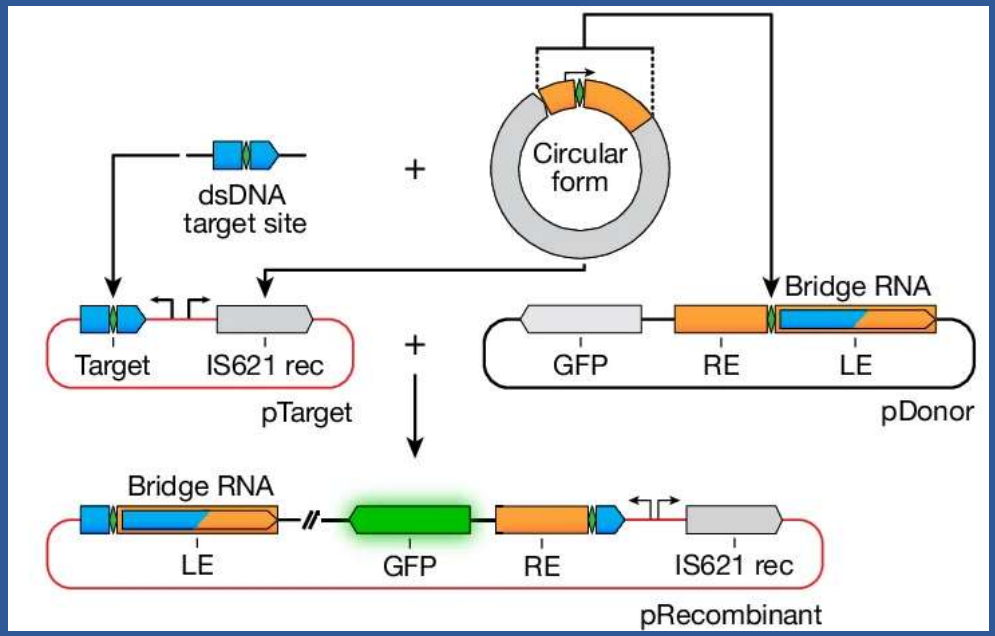


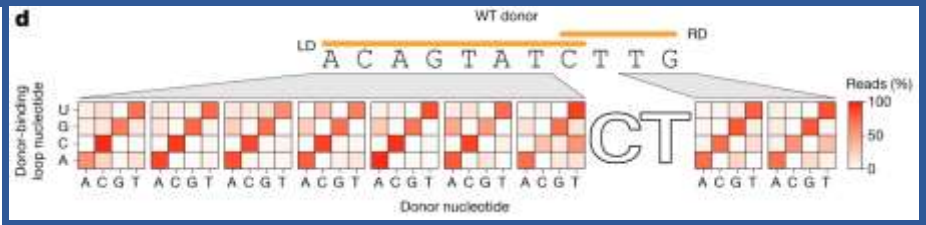

A non CRISPR with RNA programmability; recombinase not endonuclease

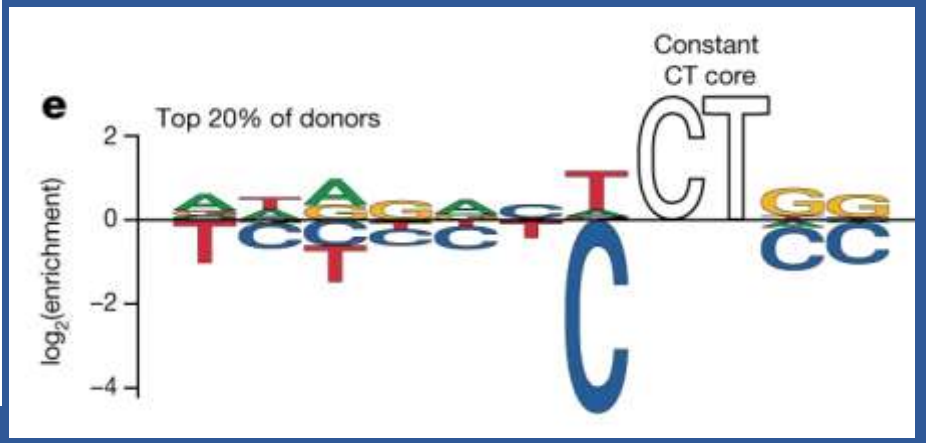
# New Tools

Durrant, M.G., et al. "Bridge RNAs direct programmable recombination of target and donor DNA." *Nature* 630, 984–993 (2024).

"We demonstrate that the target-binding and donor-binding loops can be independently reprogrammed to direct sequence-specific recombination between two DNA molecules. This modularity enables the insertion of DNA into genomic target sites, as well as programmable DNA excision and inversion."



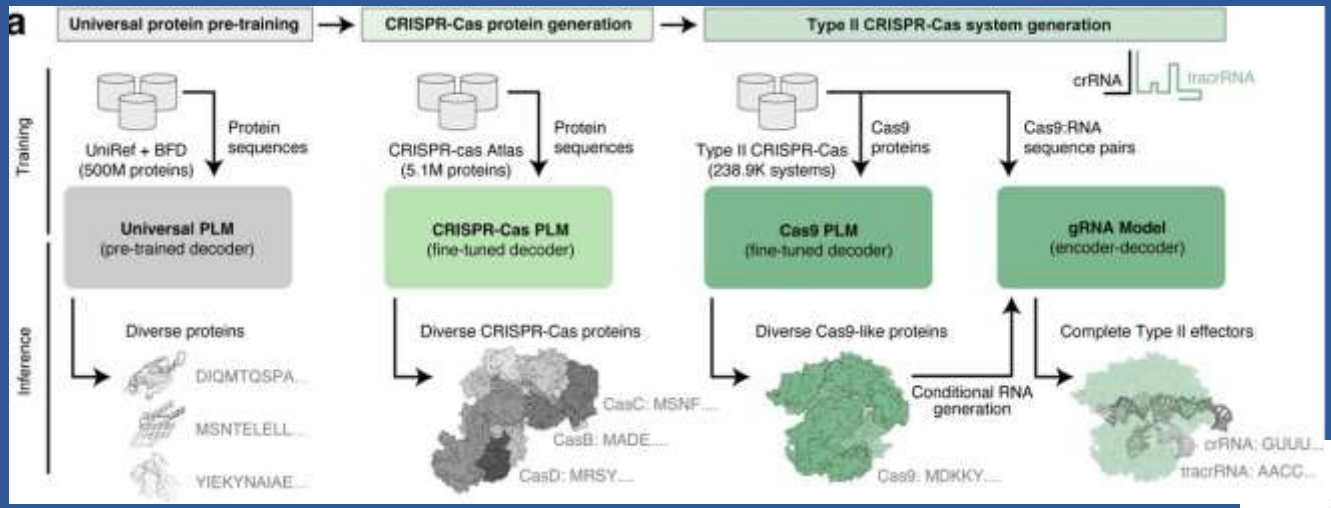




Demonstrated novel tool programmability but only bacterial genome target

# New Tools

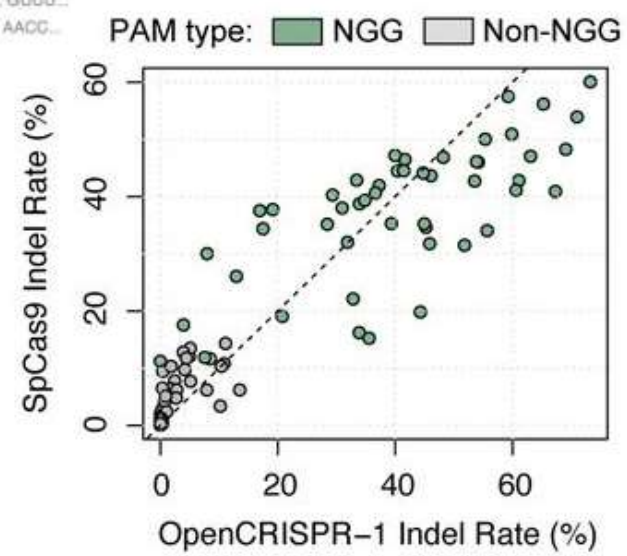
Ruffolo, Jeffrey A., et al. "Design of highly functional genome editors by modeling the universe of CRISPR-Cas sequences." *bioRxiv* (2024): 2024-04.

Using all known Cas editors as training for a “language model” developed novel editors via A.I.




OpenCRISPR is an AI-generated and functional genome editor

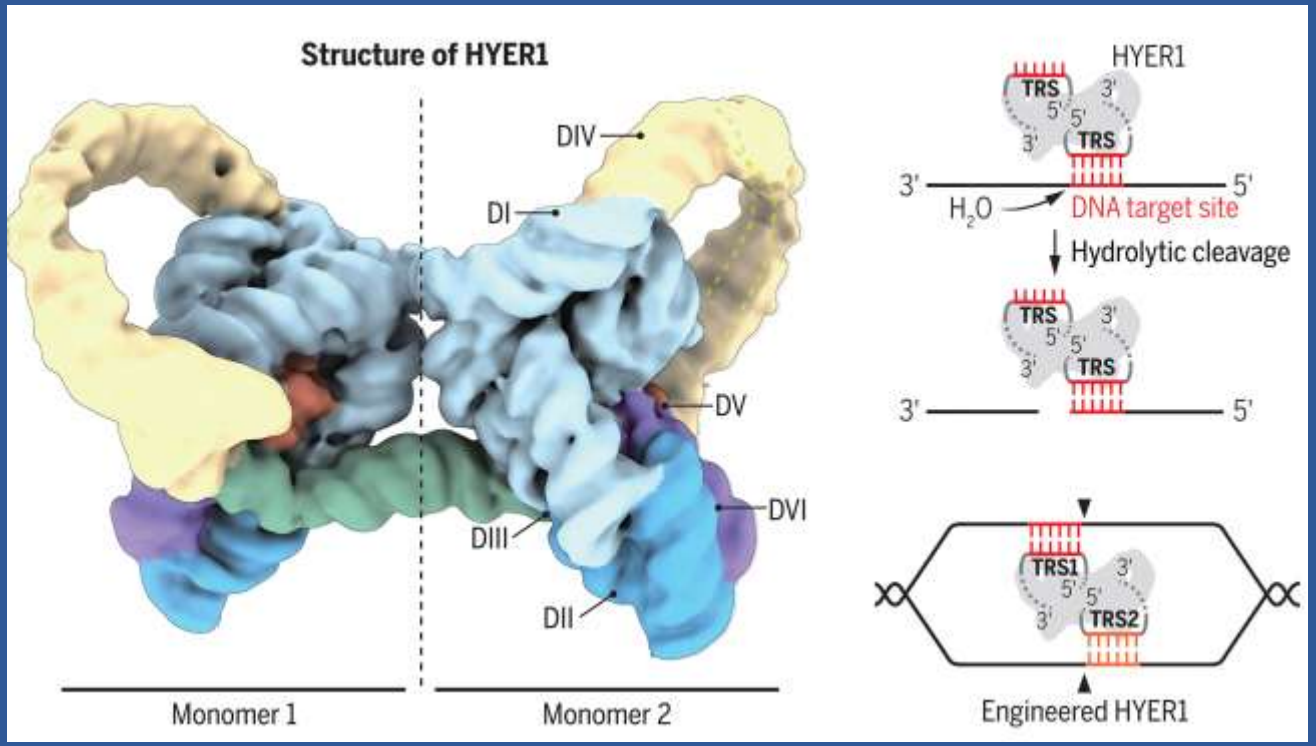
Maybe “open source” license



# New Tools

Liu, Zi-Xian, et al. "Hydrolytic endonucleolytic ribozyme (HYER) is programmable for sequence-specific DNA cleavage." *Science* 383.6682 (2024): eadh4859.

We discovered HYERs as sequence-specific and hydrolytic endonucleases. HYER1 showed plasmid interference activity in bacterial cells and genome-editing capability in mammalian cells. Cryo-EM analysis revealed the homodimer structure of HYER1, which captures the complementary DNA target via TRS and cleaves the target using the hydrolytic mechanism.



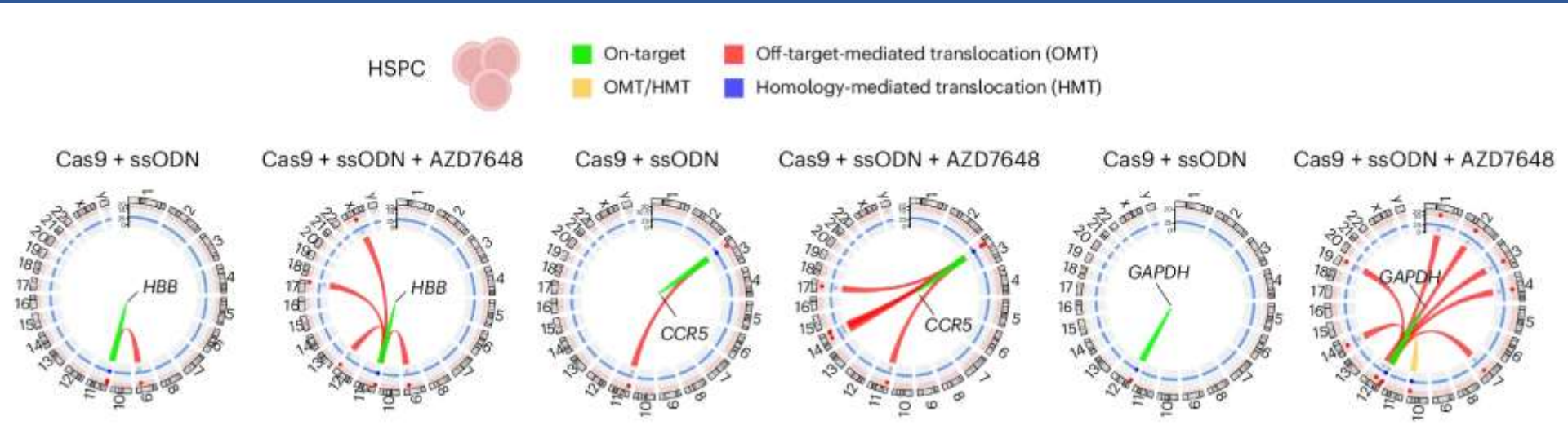

Catalytic RNA able to perform targeted cleavage of DNA.

One utility is compactness compared to protein expression.

# Cautionary Tales

Cullot, Grégoire, et al. "Genome editing with the HDR-enhancing DNA-PKcs inhibitor AZD7648 causes large-scale genomic alterations." *Nature Biotechnology* (2024): 1-5.

"editing with AZD7648 causes frequent kilobase-scale and megabase-scale deletions, chromosome arm loss and translocations. These large-scale chromosomal alterations evade detection through typical genome editing assays, prompting caution in deploying AZD7648 and reinforcing the need to investigate multiple types of potential editing outcomes"

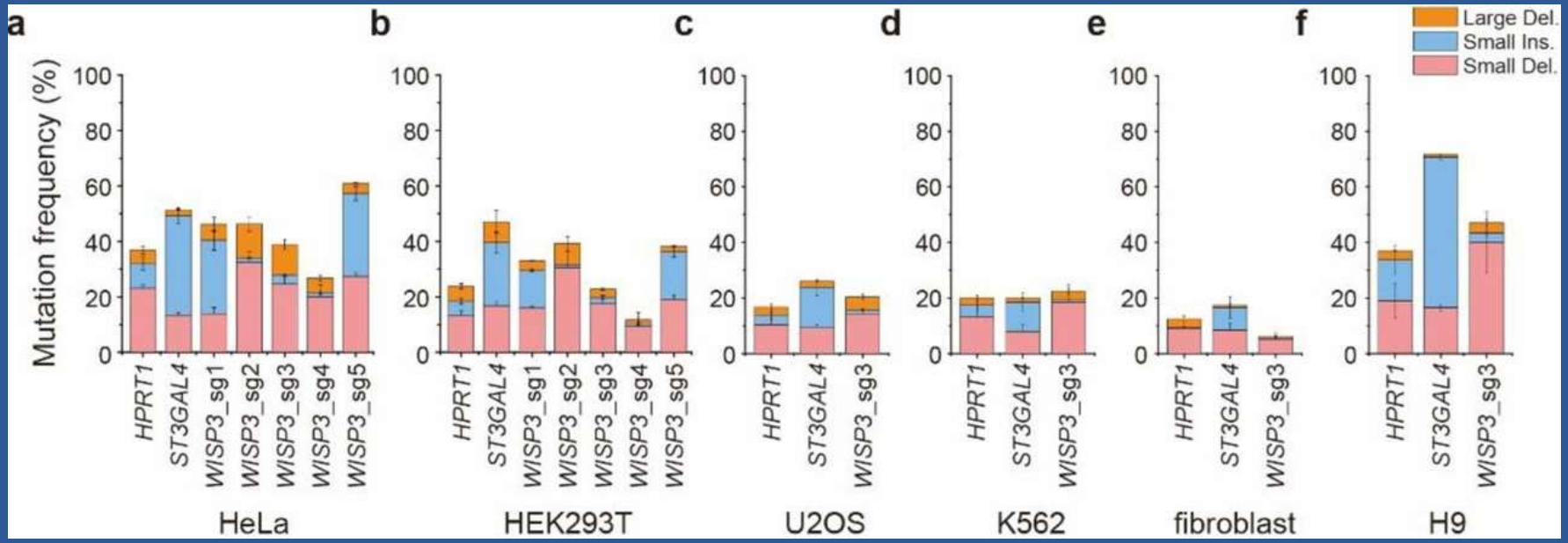



Trying to alter repair pathways to the desired homologous recombination pathway leads also to more side-effects

# Cautionary Tales

Hwang, Gue-ho, et al. "Detailed mechanisms for unintended large DNA deletions with CRISPR, base editors, and prime editors." *bioRxiv* (2024): 2024-01.

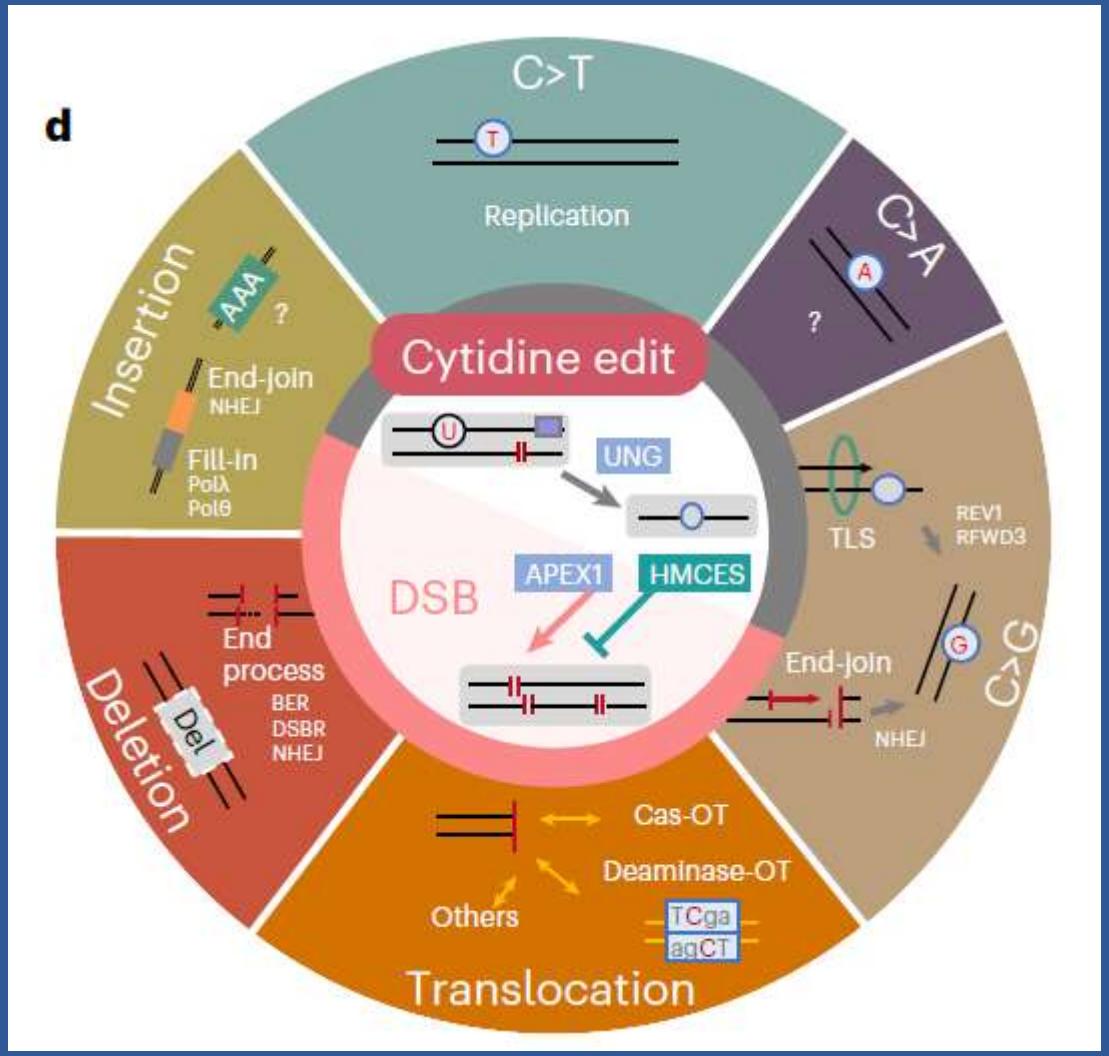
We optimized a long-range amplicon sequencing system and developed a k-mer sequence-alignment algorithm to simultaneously detect small DNA alteration events and large DNA deletions. With this workflow, we determined that CRISPR-Cas9 induced large deletions at varying frequencies in cancer cell lines, stem cells, and primary T cells. … Furthermore, base editors and prime editors also generated large deletions despite employing mutated Cas9 “nickases” that produce single-strand breaks.



# Cautionary Tales

**Huang, Min Emma, et al. "C-to-G editing generates double-strand breaks causing deletion, transversion and translocation." *Nature Cell Biology* 26.2 (2024): 294-304.**

Here, we performed in-depth outcome profiling and genetic dissection, revealing that C-to-G BEs (CGBEs) generate substantial amounts of intermediate double-strand breaks (DSBs), which are at the centre of several byproducts. Imperfect DSB end-joining leads to small deletions via end-resection, templated insertions or aberrant transversions during end fill-in. Chromosomal translocations were detected between the editing target and off-targets of Cas9/deaminase origin.



# 2024 CRISPR Year in Review

A very exciting year demonstrating

--wider pipeline of things in clinical therapy development

--more CRISPR foods and sustainability development in agriculture in US

--dramatic variety of tools for R&D and might change the IP landscape

--comprehensive analyses detect off-target