2022 CRISPR Year in Review

Science Circle
January 7th 2023

Stephen Gasior, Ph.D.
a.k.a. Stephen Xootfly
Scientist
Corteva Agriscience

2022 ~~CRISPR~~ Year in Review
2022 Genome Editing Year in Review

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2022 Genome Editing Year in Review

But even that is an insufficient title as we technically edit the transcriptome and now even proteome

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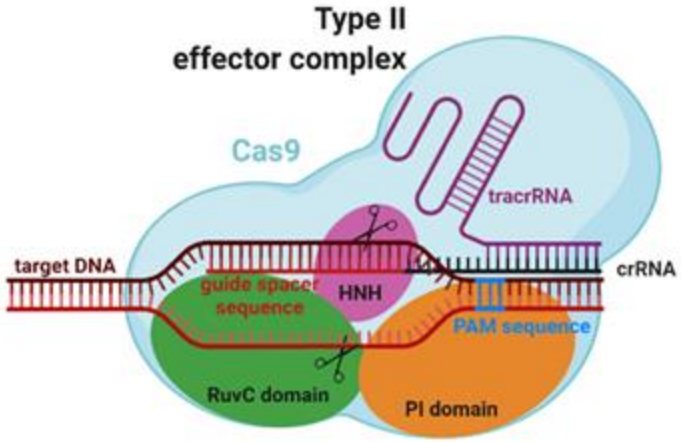
2022 Organismal Engineering Year in Review

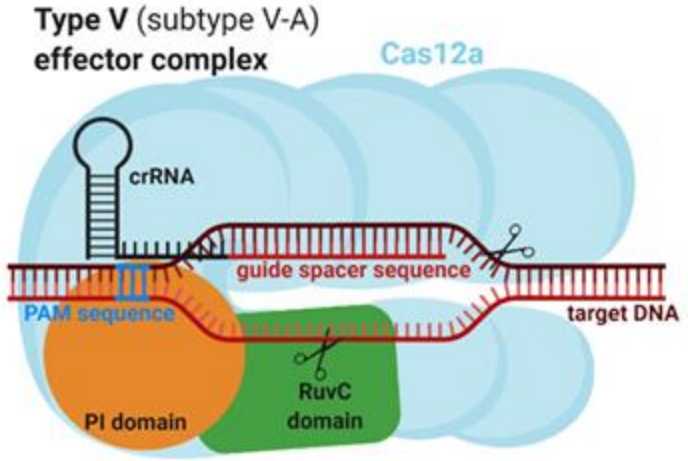
- Background
- Target Genes and Modified Organisms
- To the Consumer Advances
- Delivery Advances
- New Tools
- Technology Advances
- New Uses
- Policy and News and Media

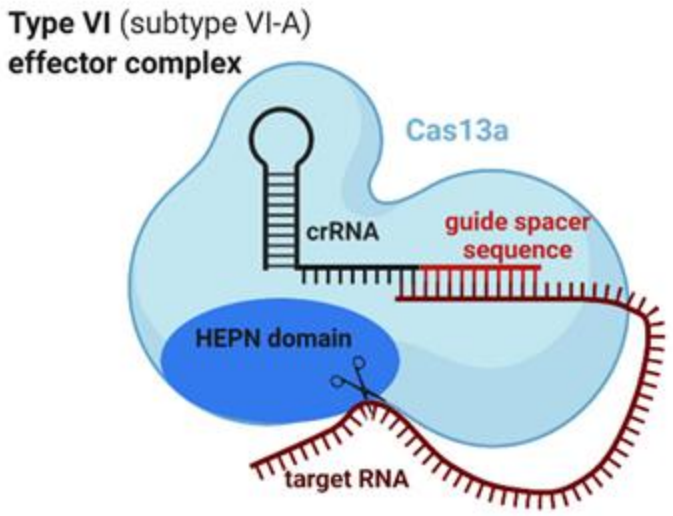
Researcher at Corteva. AgBioTech that operates in this space. Not representing the company's positions.

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

2022 Organismal Engineering Year in Review







Cas9 First and most studied and used

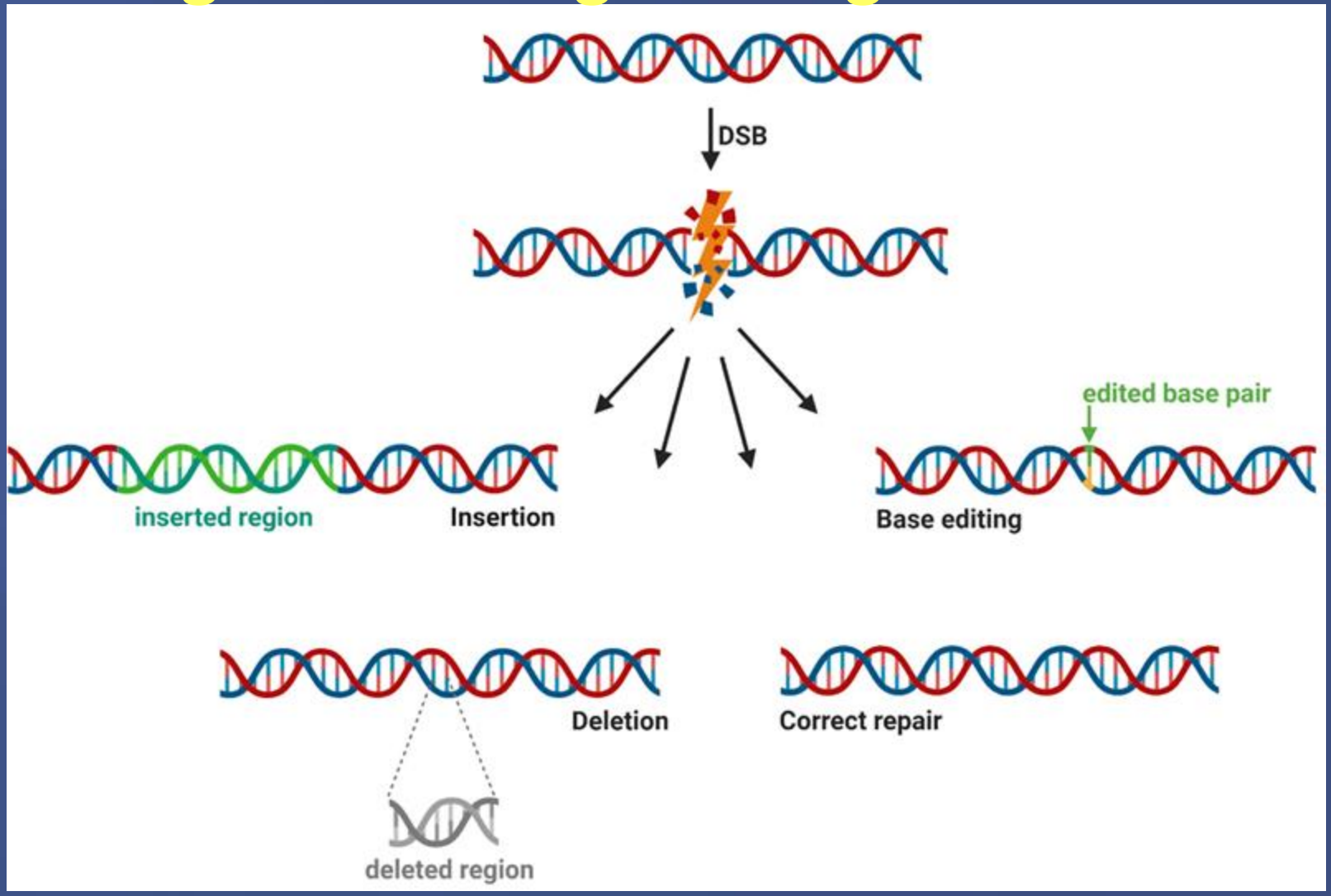
Cas12a Second most well studied (large families of Cas12s)

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.

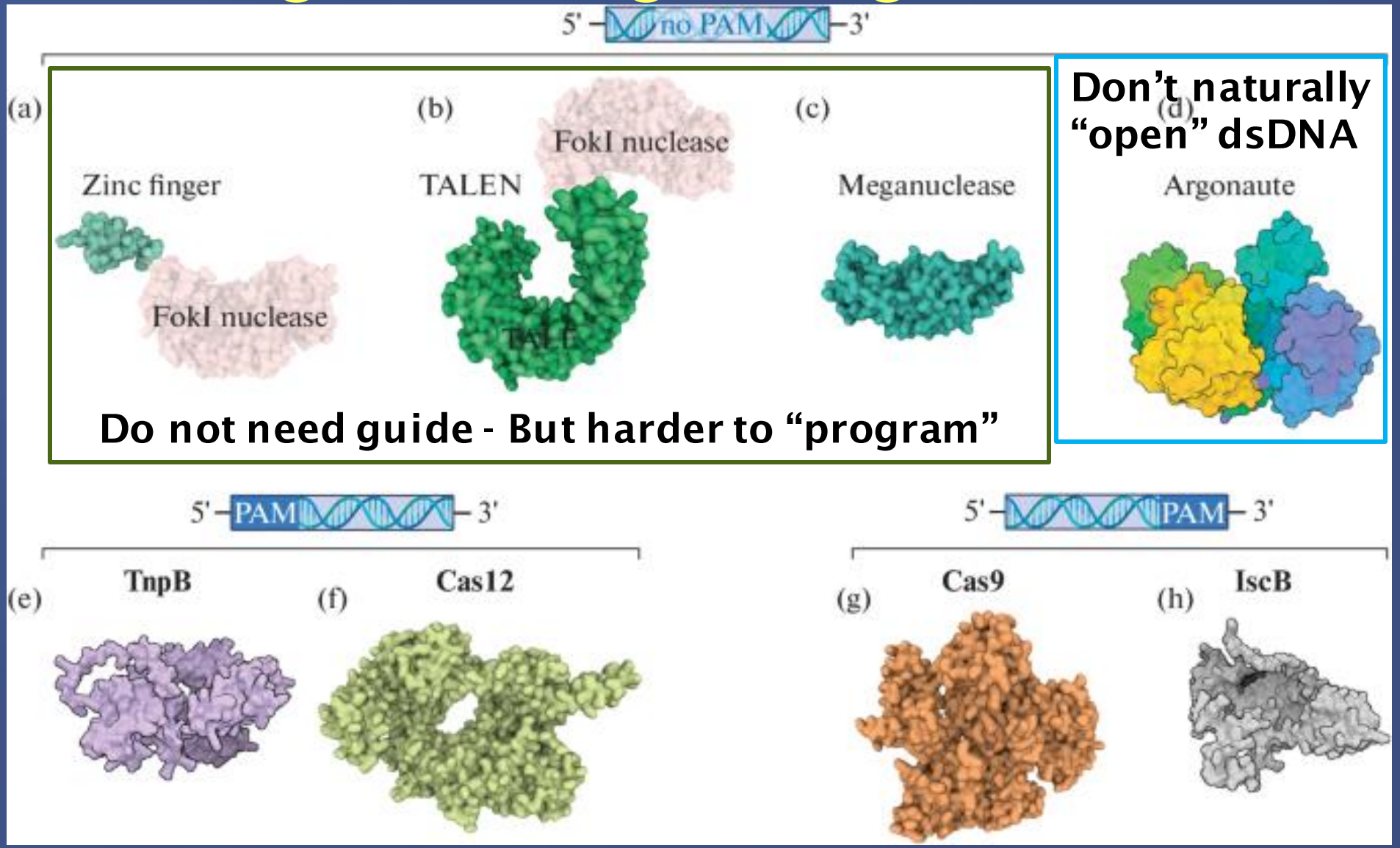
2022 Organismal Engineering Year in Review



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.

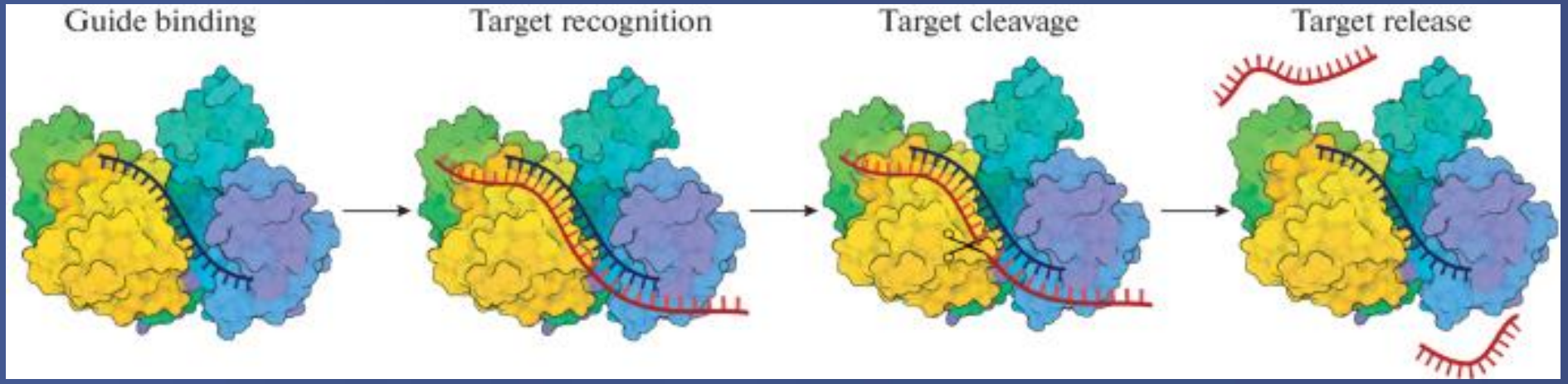
Nidhi, Sweta, et al. "Novel CRISPR–Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives." *International Journal of Molecular Sciences* 22.7 (2021): 3327.

2022 Organismal Engineering Year in Review



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

2022 Organismal Engineering Year in Review



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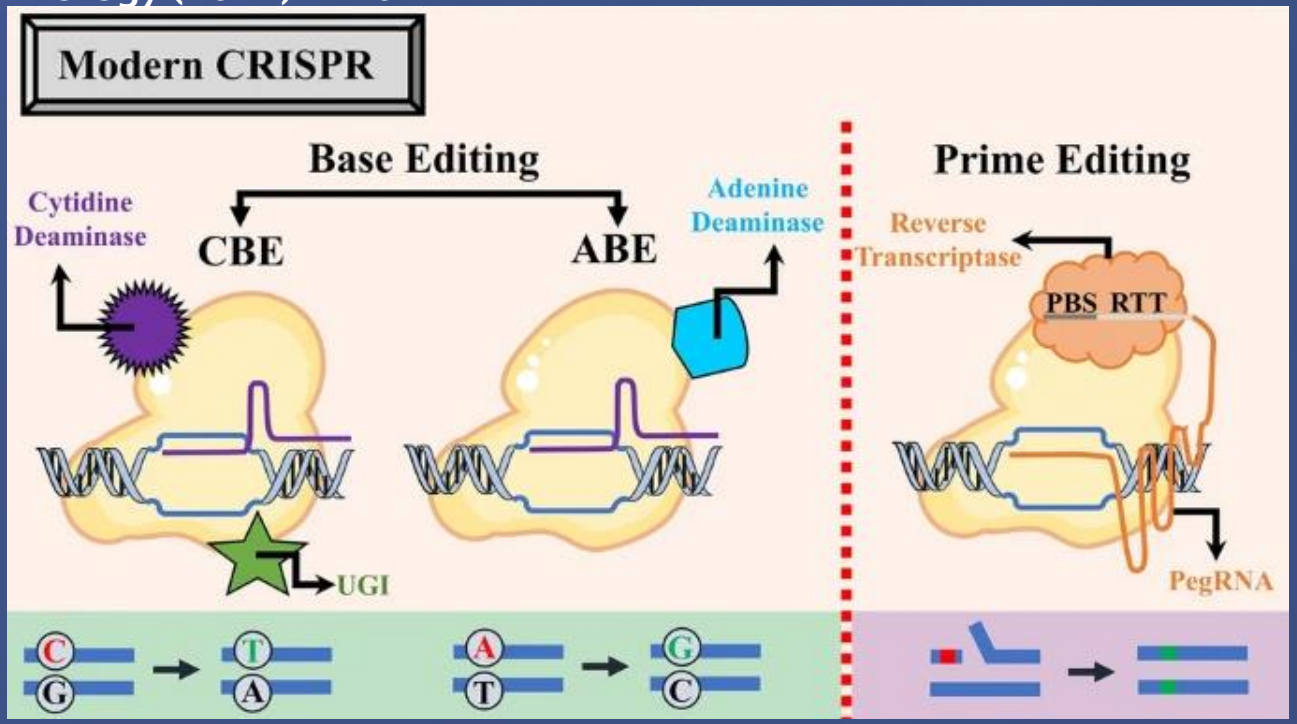
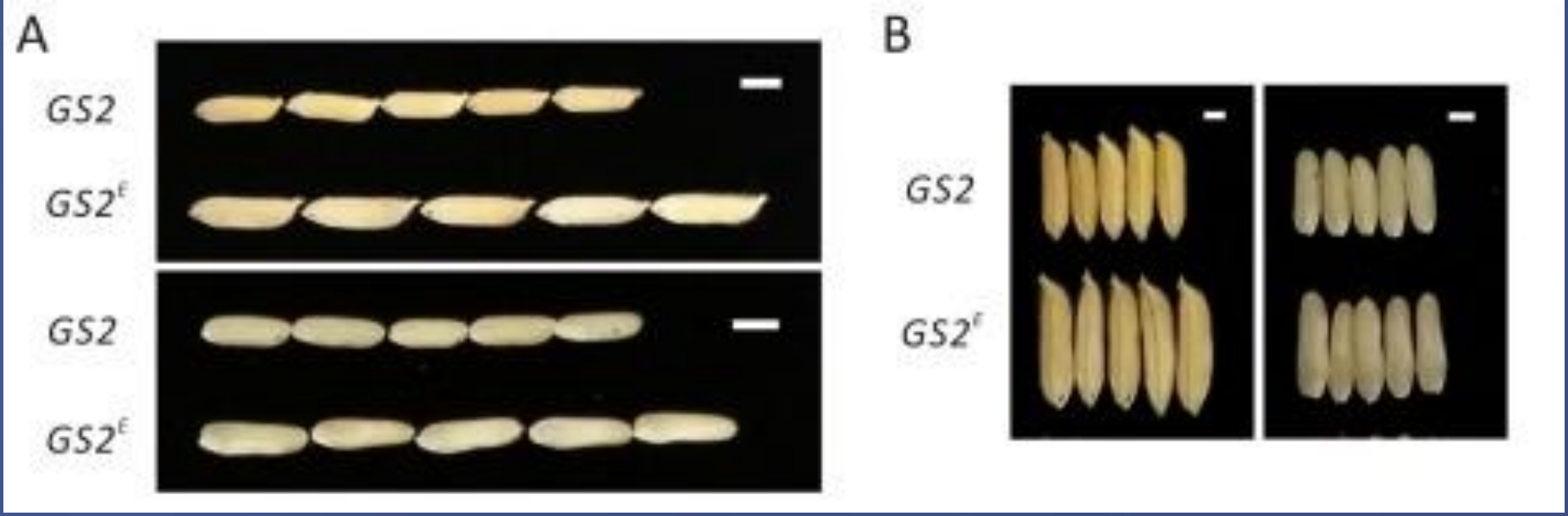


Saber Sichani, Ali, et al. "A Review on Advanced CRISPR-Based Genome-Editing Tools: Base Editing and Prime Editing." Molecular Biotechnology (2022): 1-12.

Insert novel DNA without a double-strand break

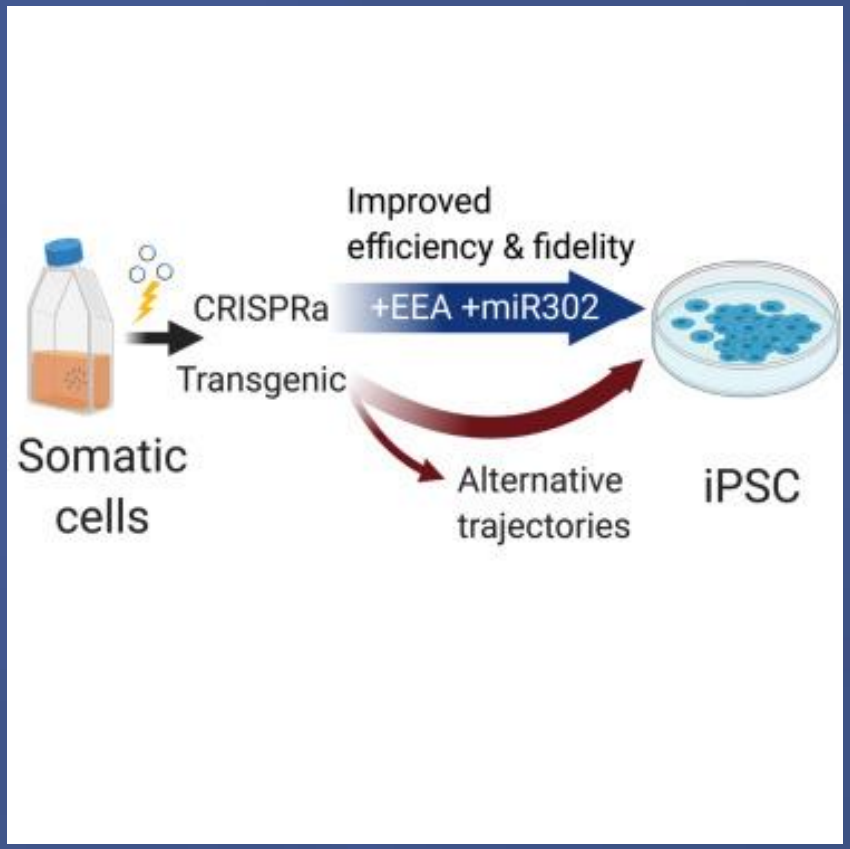
Target Genes and Modified Organisms

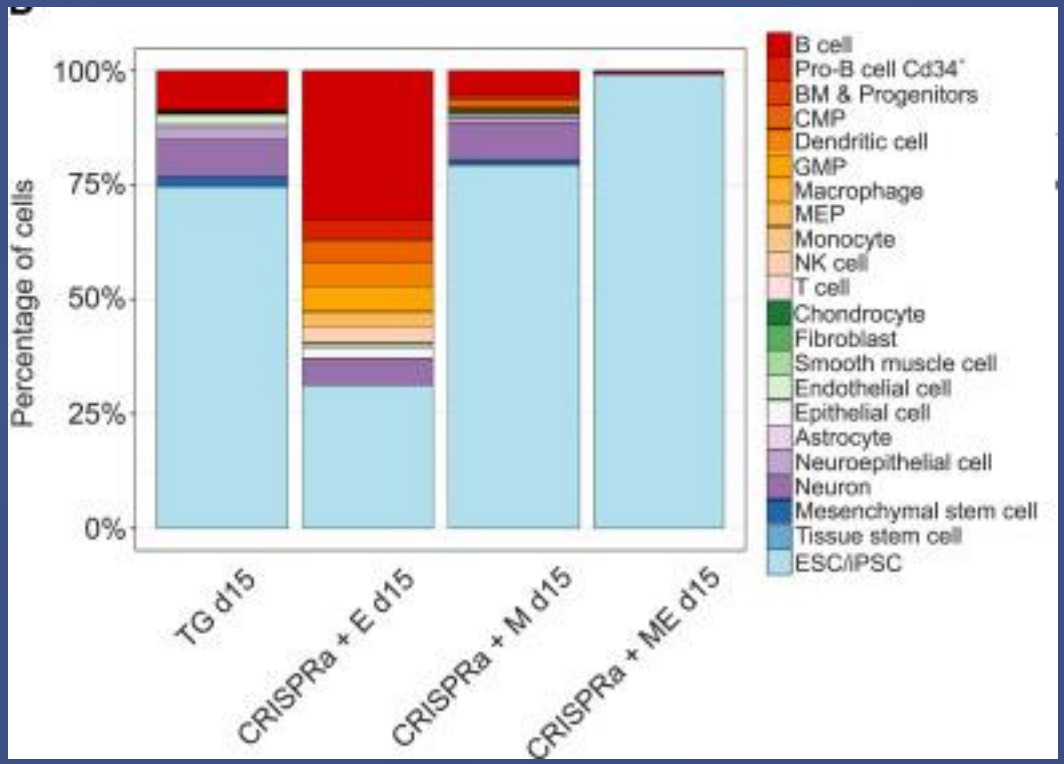


Wang, Wenshu, et al. "A new gain-of-function OsGS2/GRF4 allele generated by CRISPR/Cas9 genome editing increases rice grain size and yield." The Crop Journal (2022).

Crop genome targets remain high value and high impact for drought resistance, disease resistance, pest resistance, and increased yield

Target Genes and Modified Organisms

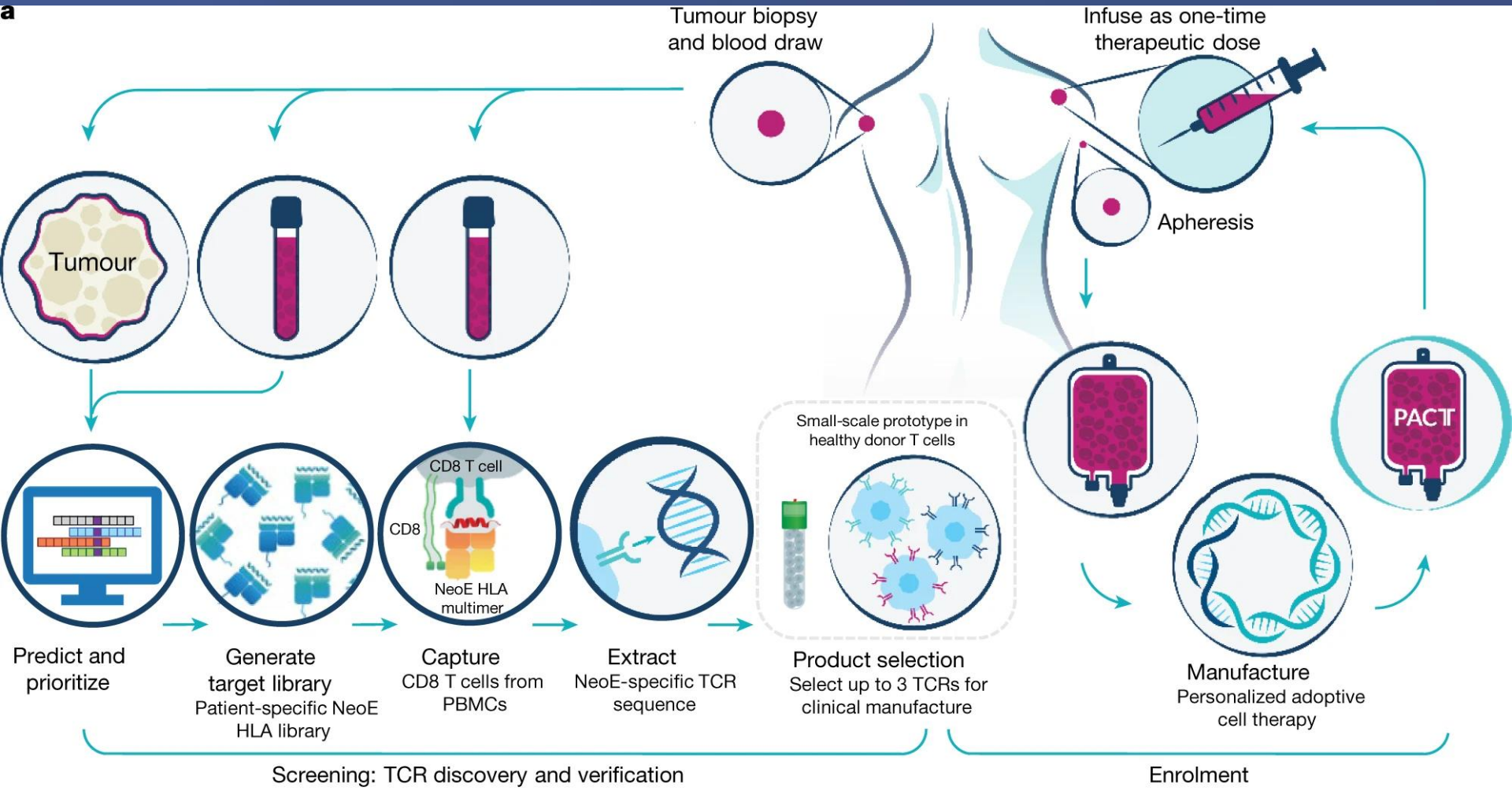




Sokka, Joonas, et al. "CRISPR activation enables high-fidelity reprogramming into human pluripotent stem cells." Stem cell reports 17.2 (2022): 413-426.

Better pluripotent stem cells aid in a variety of potential therapeutics

Target Genes and Modified Organisms

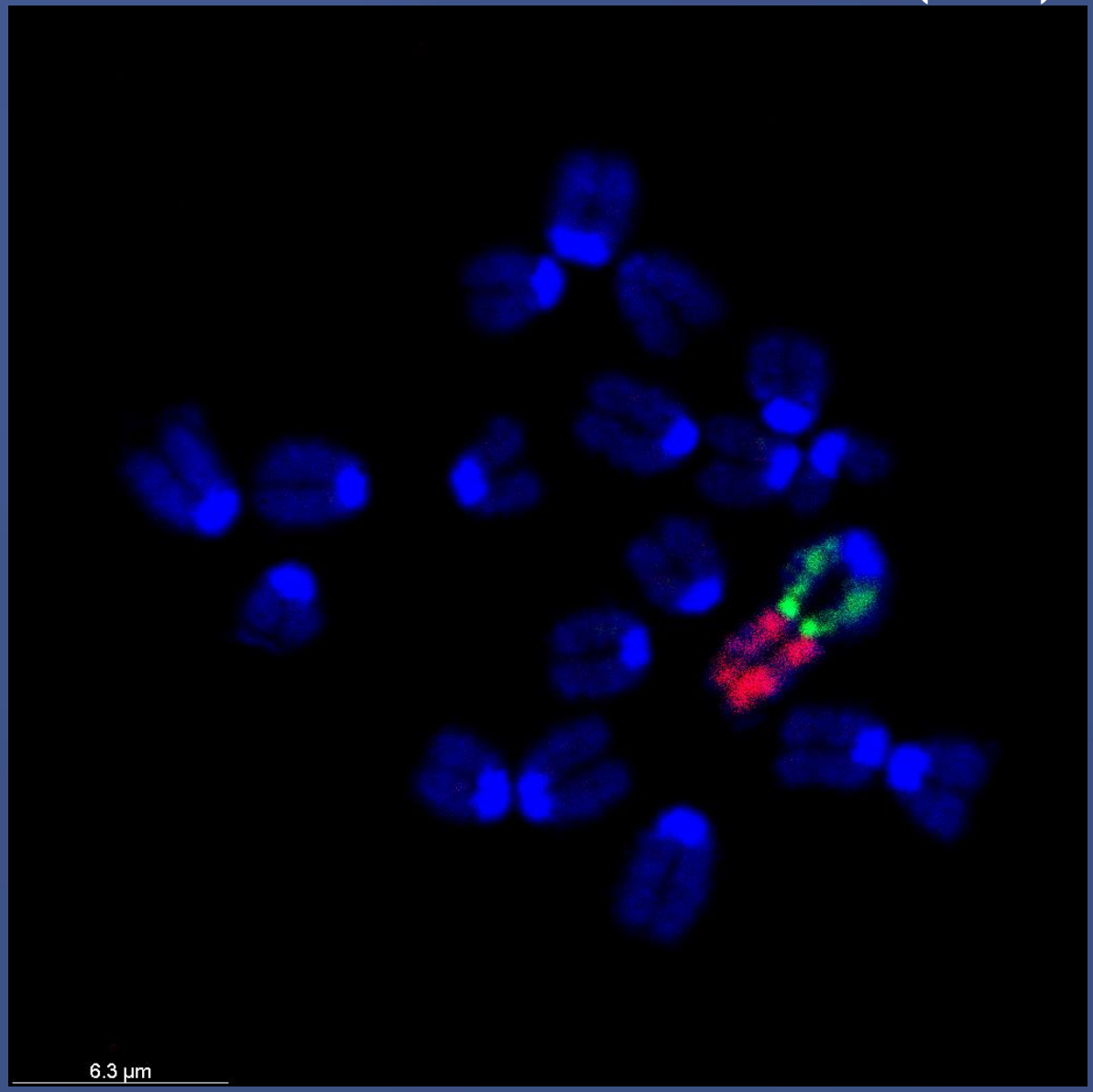


Foy, Susan P., et al. "Non-viral precision T cell receptor replacement for personalized cell therapy." Nature (2022): 1-3.

Immunotherapies as example of "personalized medicine" non-viral is novel advance to avoid immune reaction and can allow repeated use

Target Genes and Modified Organisms

Wang, Li-Bin, et al. "A sustainable mouse karyotype created by programmed chromosome fusion." Science 377.6609 (2022): 967-975.



Fused 2 chromosomes together, able to make homozygous because some birthrate was preserved

Chromosome engineering

Fixed the “imprinting” problem which has other implications….

First to human produce market

Japanese food markets … have a new distinction: people in Japan are the first consumers in the world with access to gene-edited produce.

[The GABA] tomato has found its way to thousands of home gardeners, farmers, and consumers in 2022. Produced by Tokyo-based Sanatech Seeds, these tomatoes have been edited to increase the accumulation of γ-aminobutyric acid or GABA. This compound is widely consumed as a supplement with claims of lowering blood pressure, improving mood, and increasing relaxation. While the clinical evidence supporting these claims is less than substantial, there is tremendous interest in GABA-enriched products in Japan. CRISPR-based editing of a single tomato gene produced lines with 4-20 times greater GABA levels.

MILDER MUSTARD GREENS

NON-BROWNING BANANA

VITAMIN-D RICH TOMATO

CRISPR in Agriculture: 2022 in Review
https://innovativegenomics.org/news/crispr-agriculture-2022/

First to human fish market

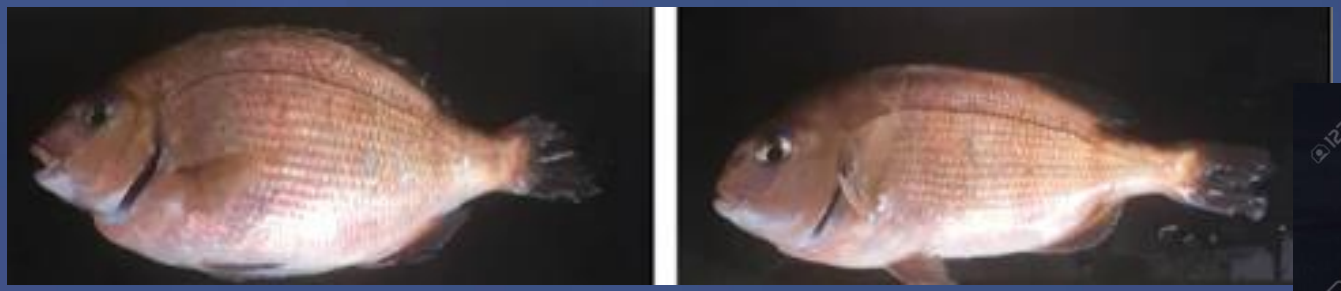
To pair with the GABA-rich tomato, a Kyoto-based start-up, in collaboration with Kyoto and Kindai Universities has developed two gene-edited fish, red sea bream and tiger puffer, now available for purchase in Japan. Both fish were CRISPR-edited to grow larger.

appetite regulation targeted so tiger puffer, ate more, and thus bigger

The red sea bream aka madai is edited in the myostatin gene, which typically limits muscle development.

Edited

Not edited



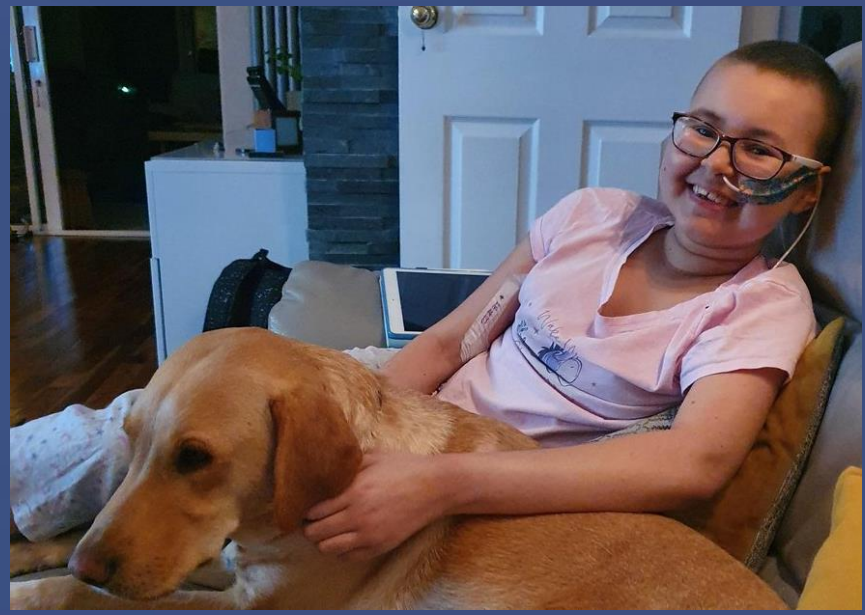
CRISPR in Agriculture: 2022 in Review
https://innovativegenomics.org/news/crispr-agriculture-2022/

Therapy Advances

Great Ormond Street Hospital for Children (GOSH) patient receives world-first treatment for her 'incurable' T-cell leukaemia

-first reported patient in the world to receive **base-edited T-cells**
-Changing the donor T-cells so that they aren't attacked by the patients own immune system
-Removing a 'flag' on the modified T-cells that means they won't attack each other before they can be used as a treatment
-Removing a second 'flag' that means the cells are invisible to other cancer treatments
-Adding a way for the modified cells to now recognise and attack cancerous T-cells

https://www.gosh.nhs.uk/news/gosh-patient-receives-world-first-treatment-for-her-incurable-t-cell-leukaemia/



https://www.youtube.com/watch?v=x4clNXVVLJw

Therapy Advances

Excision BioTherapeutics Doses First Participant in EBT-101 Phase 1/2 Trial Evaluating EBT-101 as a Potential Cure for HIV

September 15, 2022 07:00 ET | Source: Excision BioTherapeutics

EBT-101 is a unique, clinical-stage in vivo CRISPR-based therapeutic designed to cure HIV infections after a single intravenous infusion. EBT-101 employs an adeno-associated virus (AAV) to deliver CRISPR-Cas9 and dual guide RNAs, enabling a multiplex editing approach that simultaneously targets three distinct sites within the HIV genome. This allows for the excision of large portions of the HIV genome, thereby minimizing potential viral escape.

https://www.globenewswire.com/news-release/2022/09/15/2516733/0/en/Excision-BioTherapeutics-Doses-First-Participant-in-EBT-101-Phase-1-2-Trial-Evaluating-EBT-101-as-a-Potential-Cure-for-HIV.html

Generalized treatment for integrating retroviruses

Therapy Advances

Phase 1 clinical trial of CRISPR-engineered CAR19 universal T cells for treatment of children with refractory B cell leukemia

used CRISPR-Cas9 editing to disrupt T cell receptor α chain and remove CD52 in CAR19 T cells (TT52CAR19 T cells) to create a universal cell therapy

Ottaviano, Giorgio, et al. "Phase 1 clinical trial of CRISPR-engineered CAR19 universal T cells for treatment of children with refractory B cell leukemia." Science Translational Medicine 14.668 (2022): eabq3010.

Steps toward a universal donor therapy
[editorializing: we have the technology to make universal donor blood cells for decades and has yet to get to market]

Therapy Advances

CRISPR Therapeutics and ViaCyte, Inc. Announce First Patient Dosed in Phase 1 Clinical Trial of Novel Gene-Edited Cell Replacement Therapy for Treatment of Type 1 Diabetes (T1D)

VCTX210 is an investigational, allogeneic, gene-edited, stem cell-derived product developed in collaboration by applying CRISPR Therapeutics' gene-editing technology to ViaCyte's proprietary stem cell capabilities for the generation of pancreatic cells designed to evade recognition by the immune system. This immune-evasive cell replacement therapy is designed to enable patients to produce their own insulin.

https://viacyte.com/press-releases

Generalized treatment strategy for autoimmune disease

Therapy Advances

Edits to a cholesterol gene could stop the biggest killer on earth
In a first, a patient in New Zealand has undergone gene-editing to lower their cholesterol. It could be the beginning of new era in disease prevention.

By Antonio Regalado
July 12, 2022

https://www.technologyreview.com/2022/07/12/1055773/crispr-gene-editing-cholesterol/

Think about the Lipitor market share
NOT therapy per se, cholesterol lowering is prophylactic to heart disease

Therapy Advances

First pig-to-human heart transplant: what can scientists learn?

Surgeons at the University of Maryland Medical Center transplanted a genetically altered pig heart into David Bennett. Credit: University of Maryland School of Medicine

The first person to receive a transplanted heart from a genetically modified pig is doing well after the procedure last week in Baltimore, Maryland. Transplant surgeons hope the advance will enable them to give more people animal organs, but many ethical and technical hurdles remain.

Xenotransplantation has seen significant advances in recent years with the advent of CRISPR–Cas9 genome editing, which made it easier to create pig organs that are less likely to be attacked by human immune systems. The latest transplant, performed at the University of Maryland Medical Center (UMMC), used organs from pigs with ten genetic modifications.

Kind of IMPORTANT UPDATE:

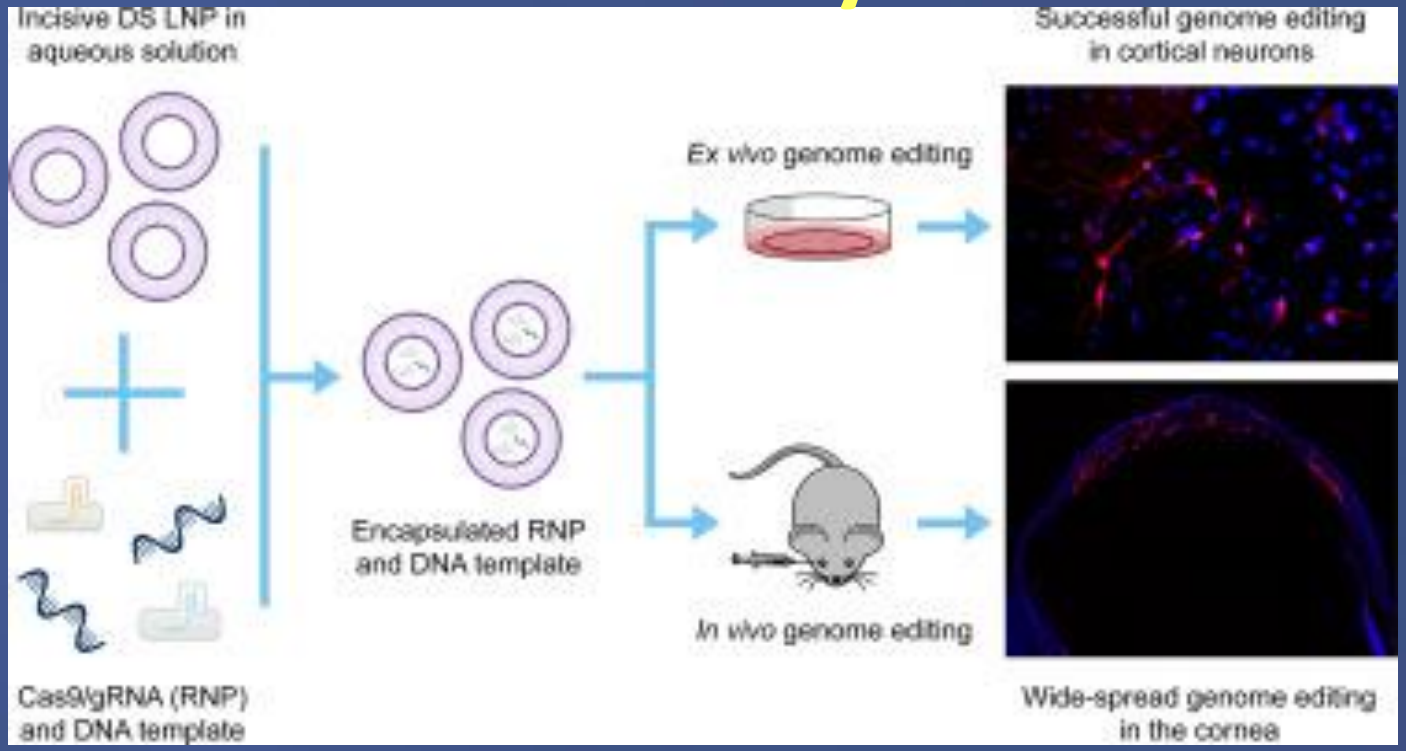
Bennett survived for eight weeks with his new heart before his body shut down. After his death, the research team learnt that the transplanted organ was infected with a pig herpesvirus that had not been detected by tests.

https://www.nature.com/articles/d41586-022-00111-9

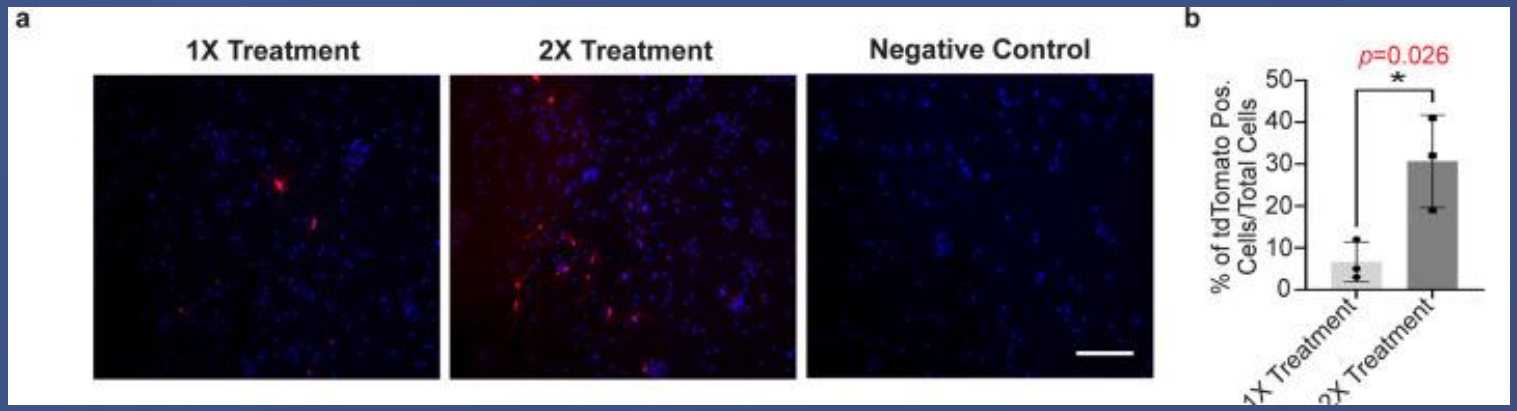
https://www.nature.com/articles/d41586-022-03794-2

Still lots and lots of hurdles for long term therapy

Delivery Advances

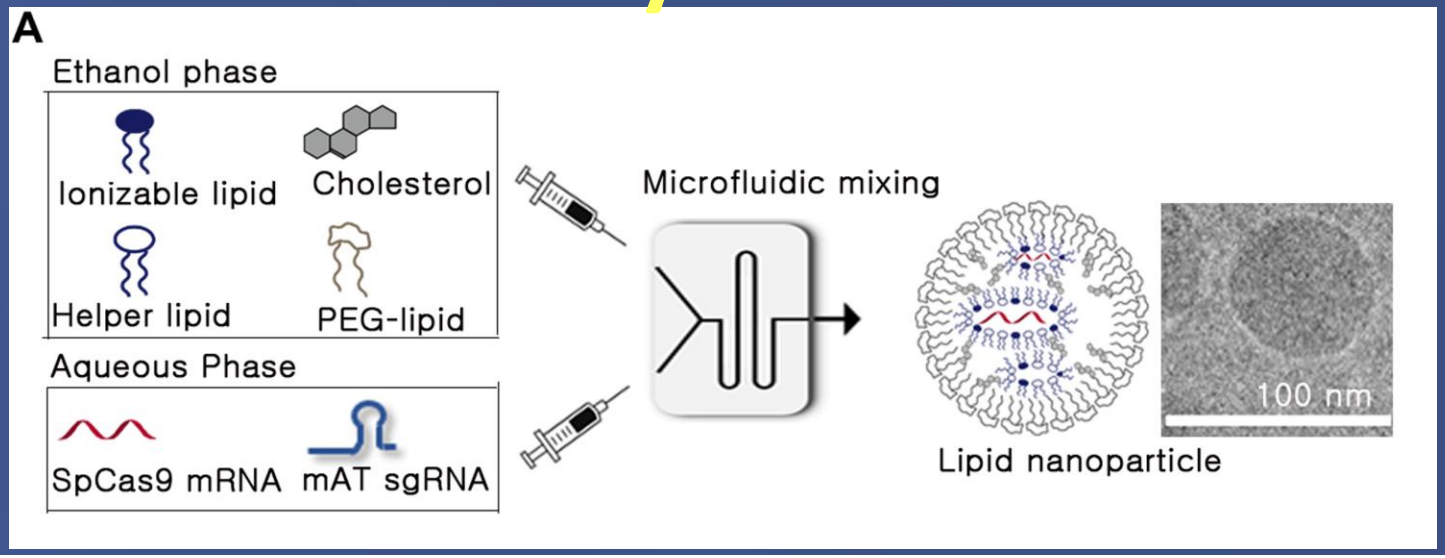


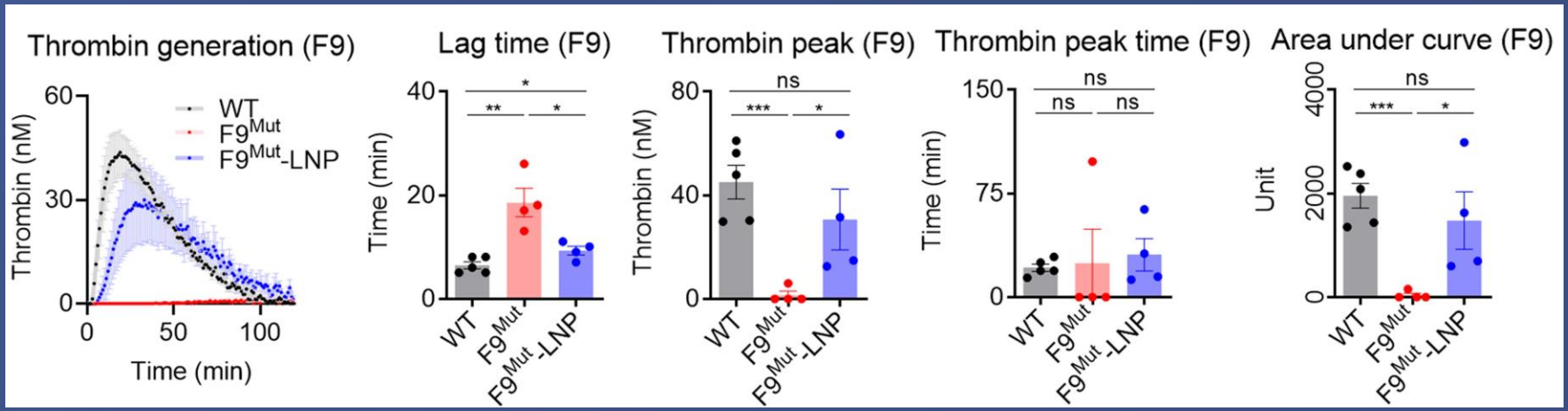
Specialized surface tissues have lower threshold for delivery



Mohanna, Seyedeh Zeinab Mirjalili, et al. "LNP-mediated delivery of CRISPR RNP for wide-spread in vivo genome editing in mouse cornea." Journal of Controlled Release 350 (2022): 401-413.

Delivery Advances

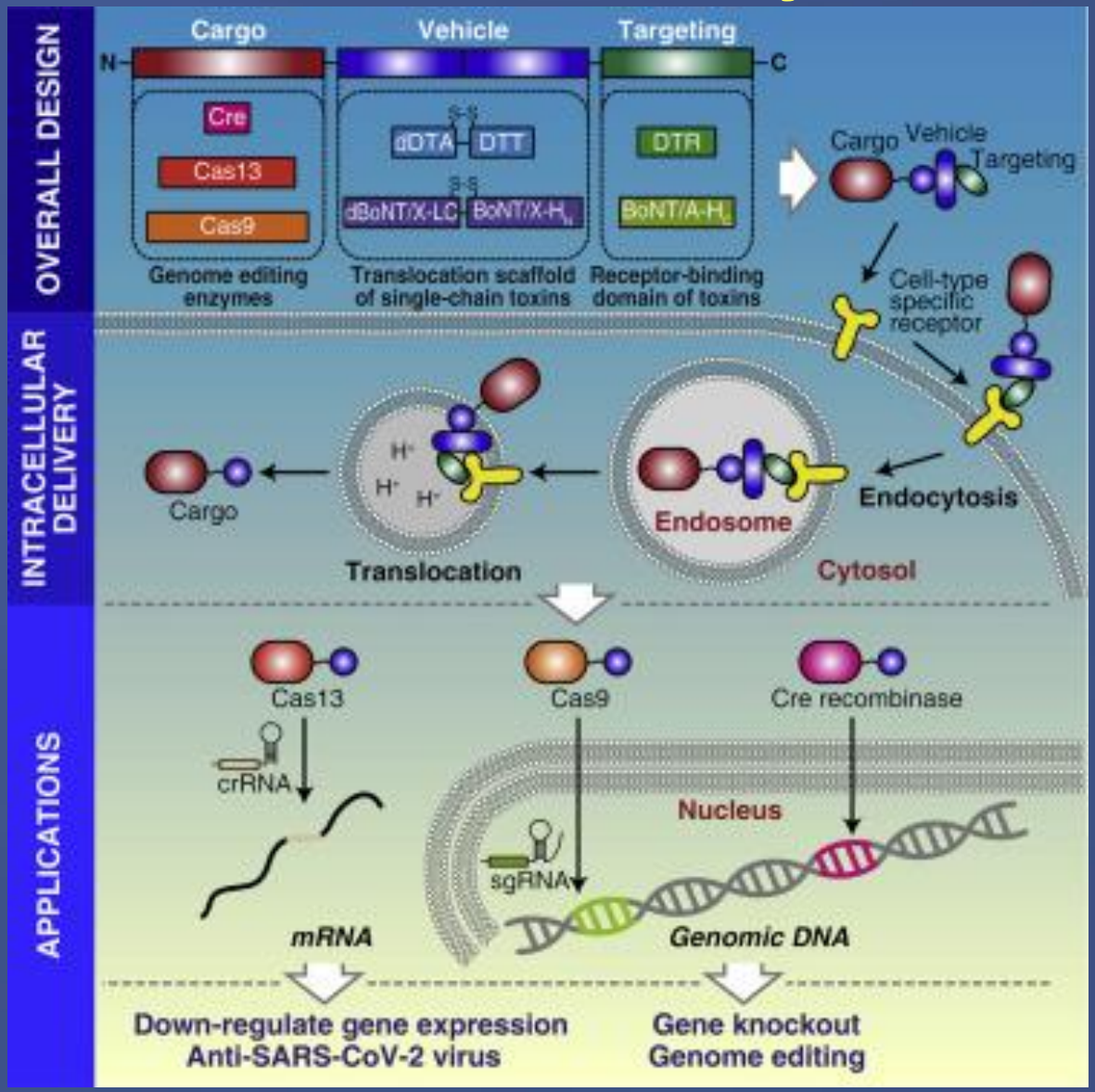




Same as previous – highly targetable tissue (liver) but then widespread effect: enhanced clotting throughout body

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.

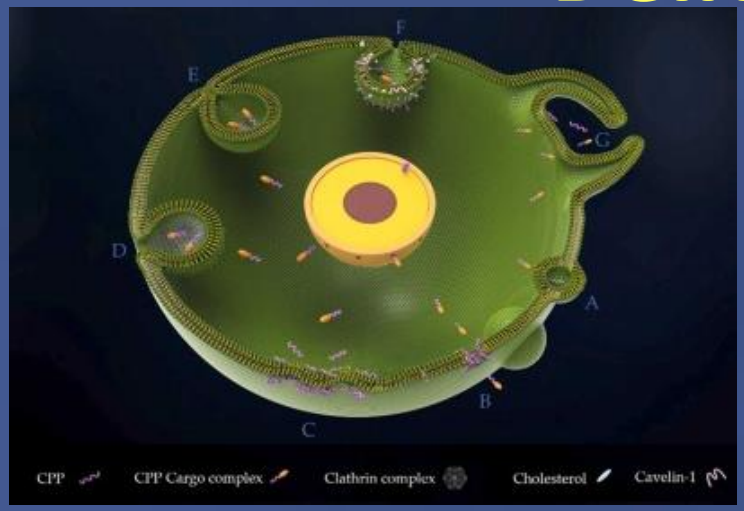
Delivery Advances

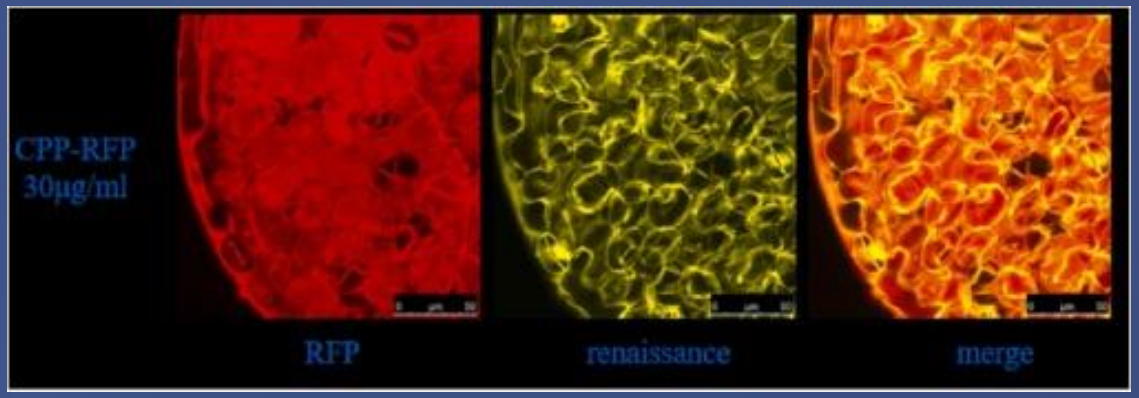


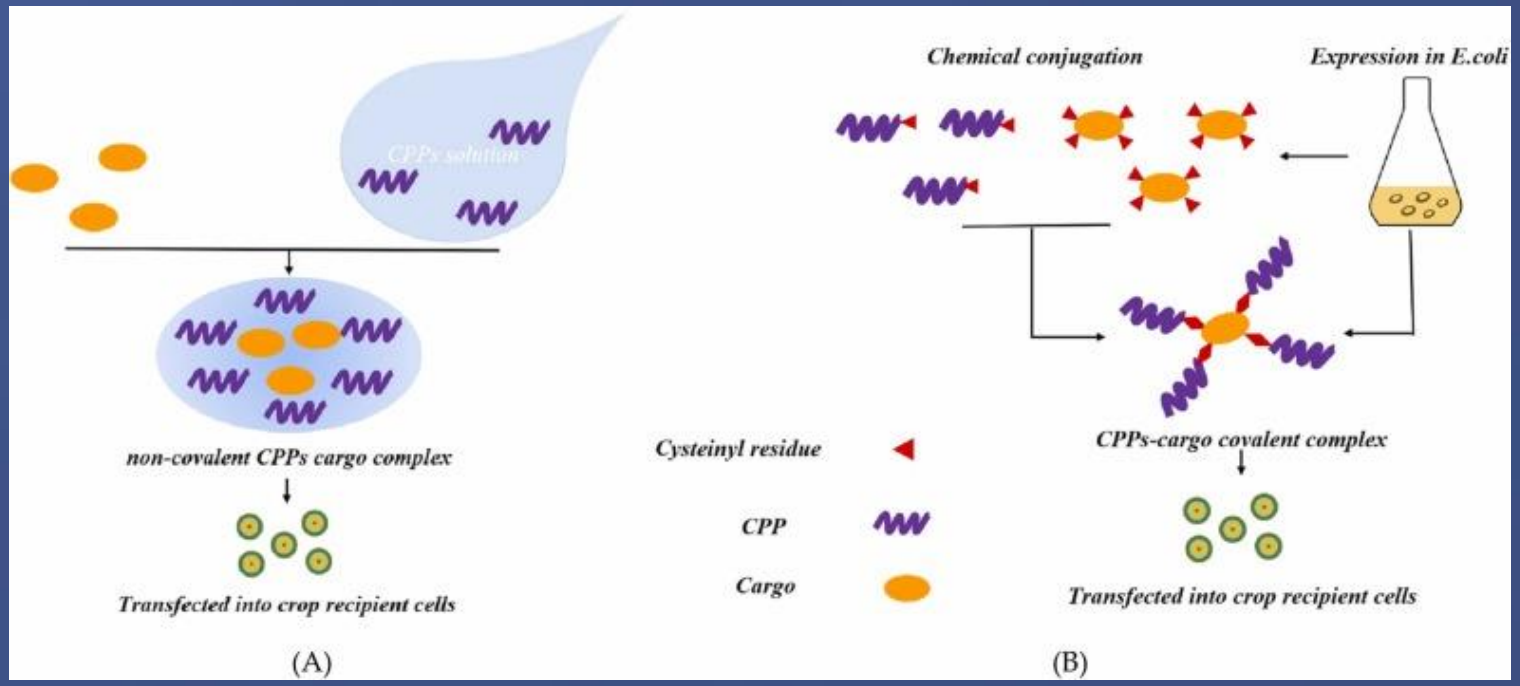
Similar to previous – accessible tissue but no lipid coating

Tian, Songhai, et al. "Targeted intracellular delivery of Cas13 and Cas9 nucleases using bacterial toxin-based platforms." Cell reports 38.10 (2022): 110476.

Delivery Advances



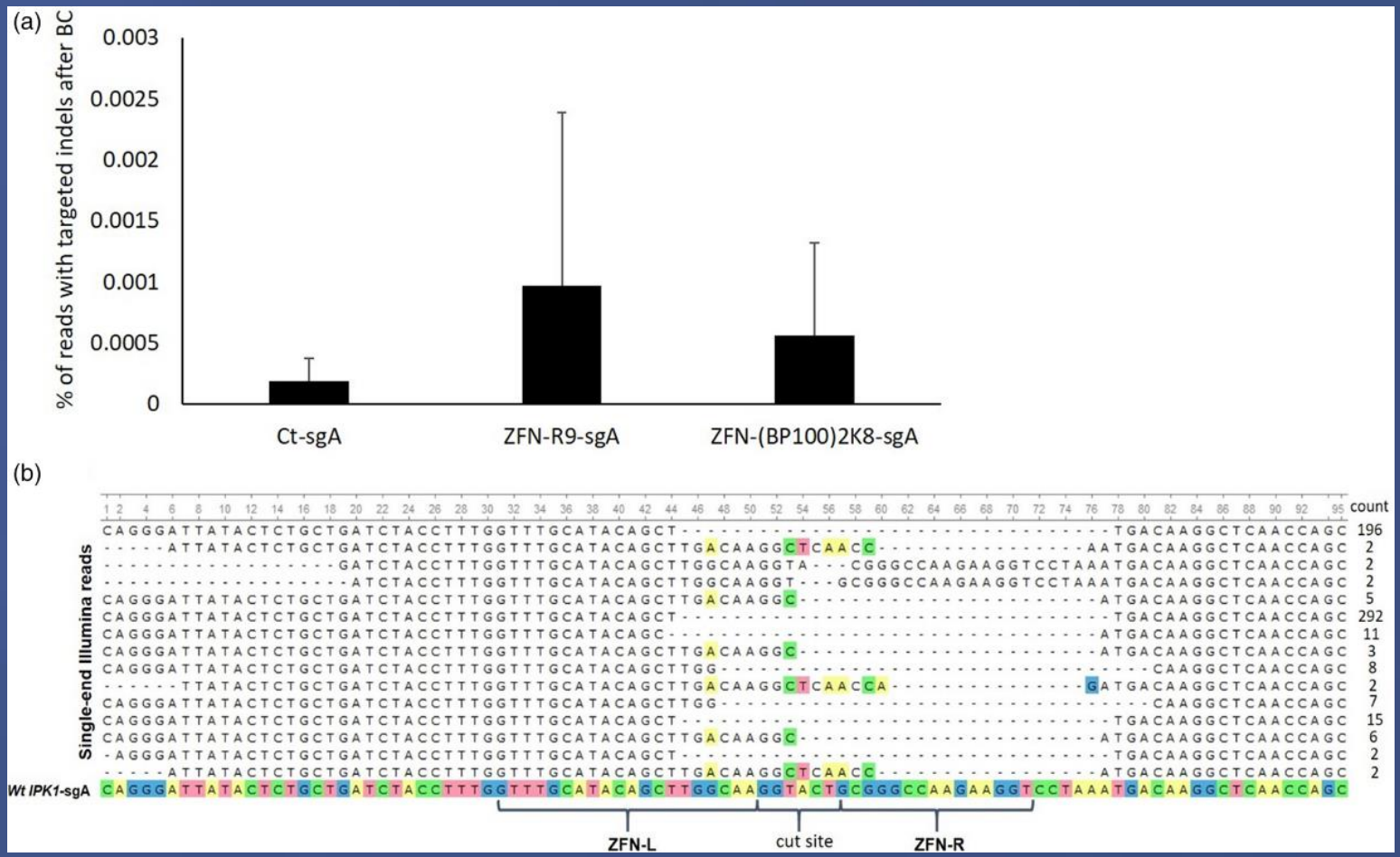




Eventually, the new crop dusting?

Wu, Han, et al. "Cell-penetrating peptide: A powerful delivery tool for DNA-free crop genome editing." Plant Science (2022): 111436.

Delivery Advances

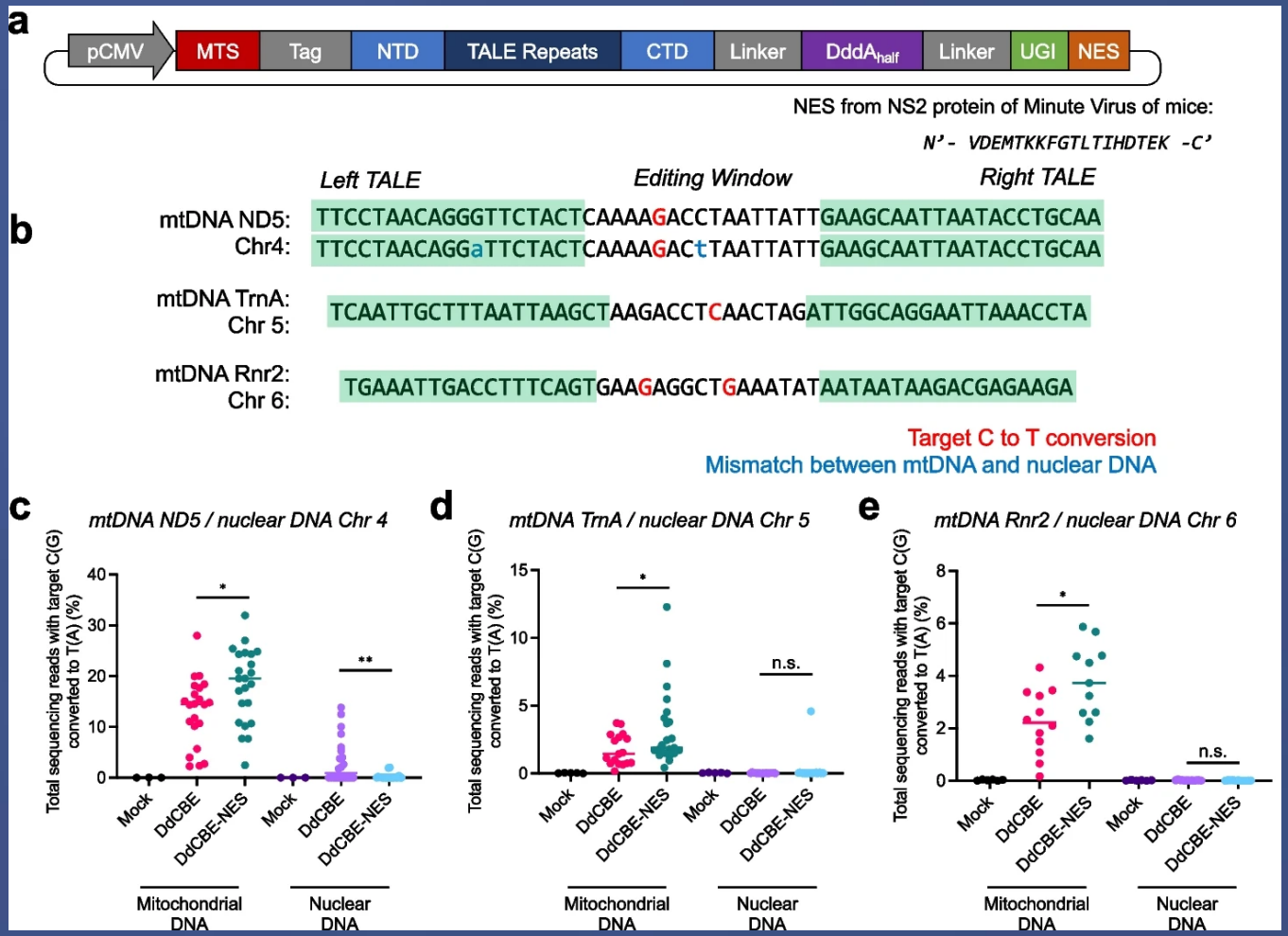


Bilichak, Andriy, et al. "Genome editing in wheat microspores and haploid embryos mediated by delivery of ZFN proteins and cell-penetrating peptide complexes." Plant biotechnology journal 18.5 (2020): 1307-1316.

Using a ZFN, NOT CRISPR, only need to deliver protein, so cell penetrating peptides use for everything you need

Delivery Advances

Lee, Seonghyun, et al. "Precision mitochondrial DNA editing with high-fidelity DddA-derived base editors." Nature Biotechnology (2022): 1-9.

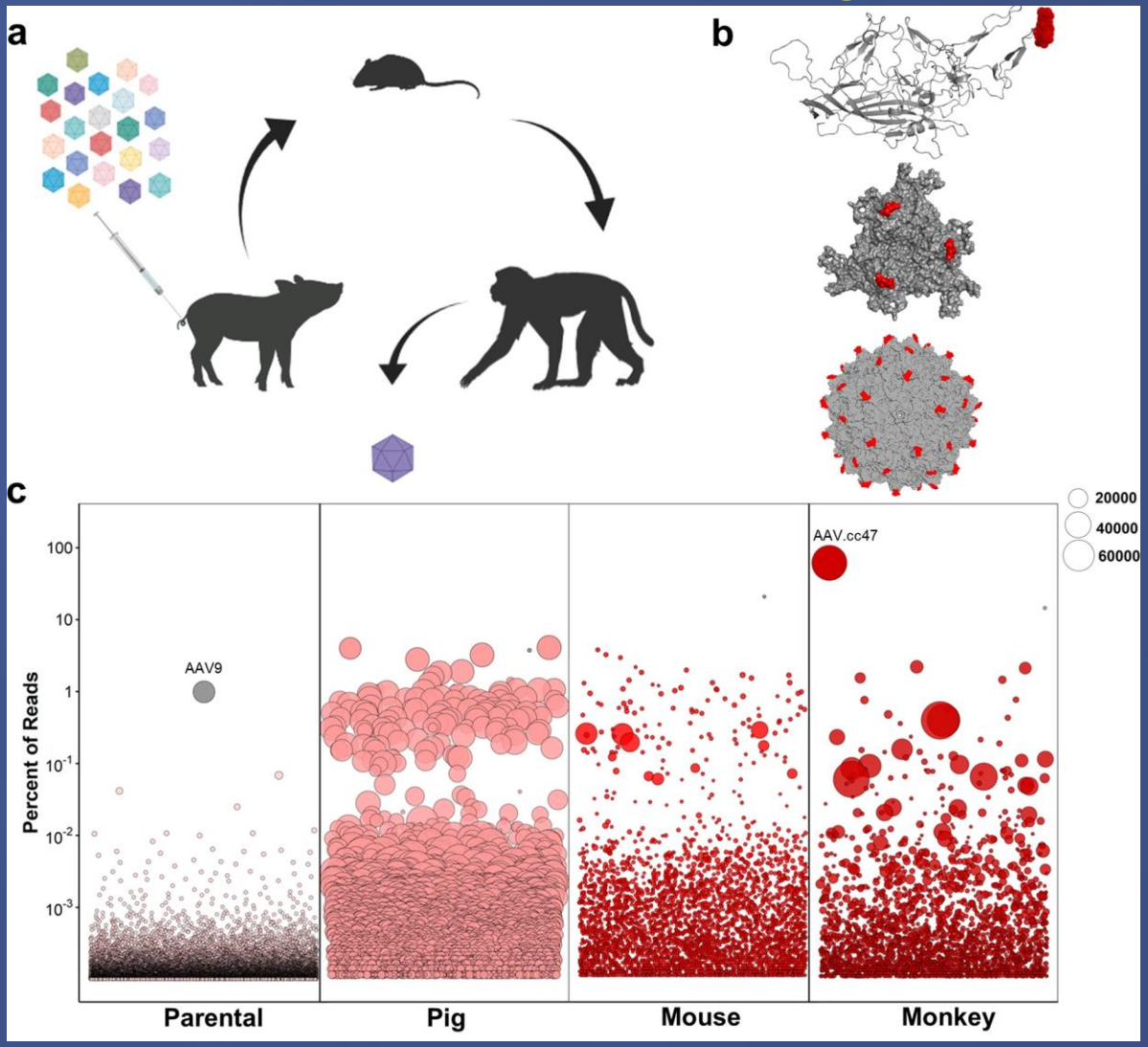


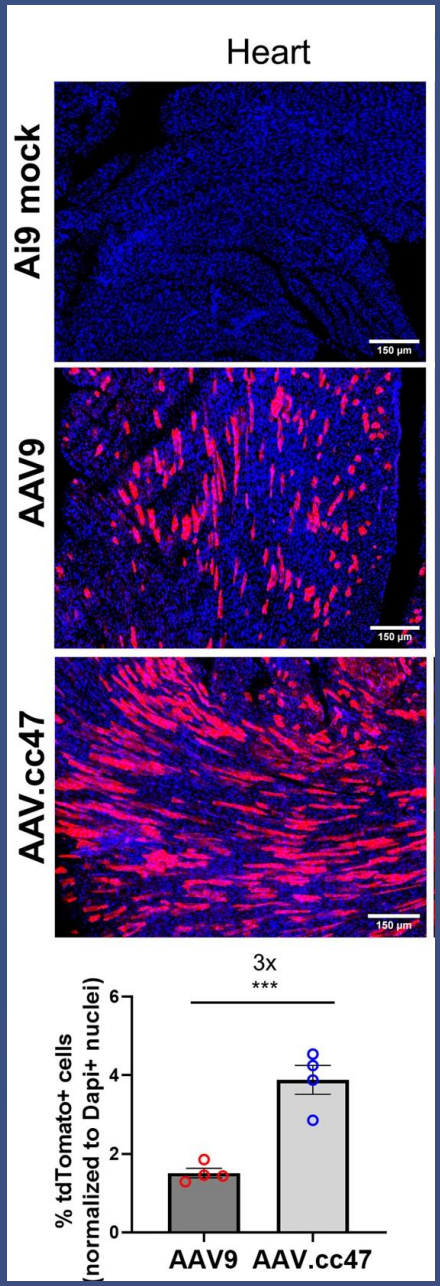
Subcellular organelle targeting – mitochondria

NON CRISPR so doesn't require RNA delivery

(related) Lim, Kayeong, Sung-Ik Cho, and Jin-Soo Kim. "Nuclear and mitochondrial DNA editing in human cells with zinc finger deaminases." Nature communications 13.1 (2022): 1-10.

Delivery Advances

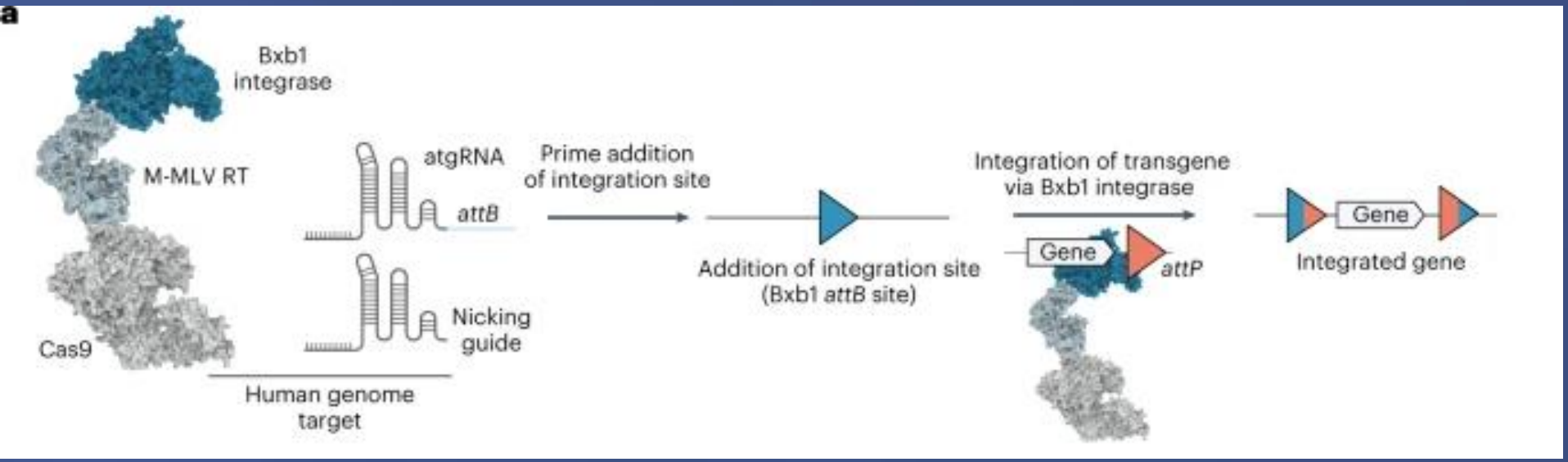




Evolving the AAV virus

Gonzalez, Trevor J., et al. "Cross-species evolution of a highly potent AAV variant for therapeutic gene transfer and genome editing." Nature Communications 13.1 (2022): 1-17.

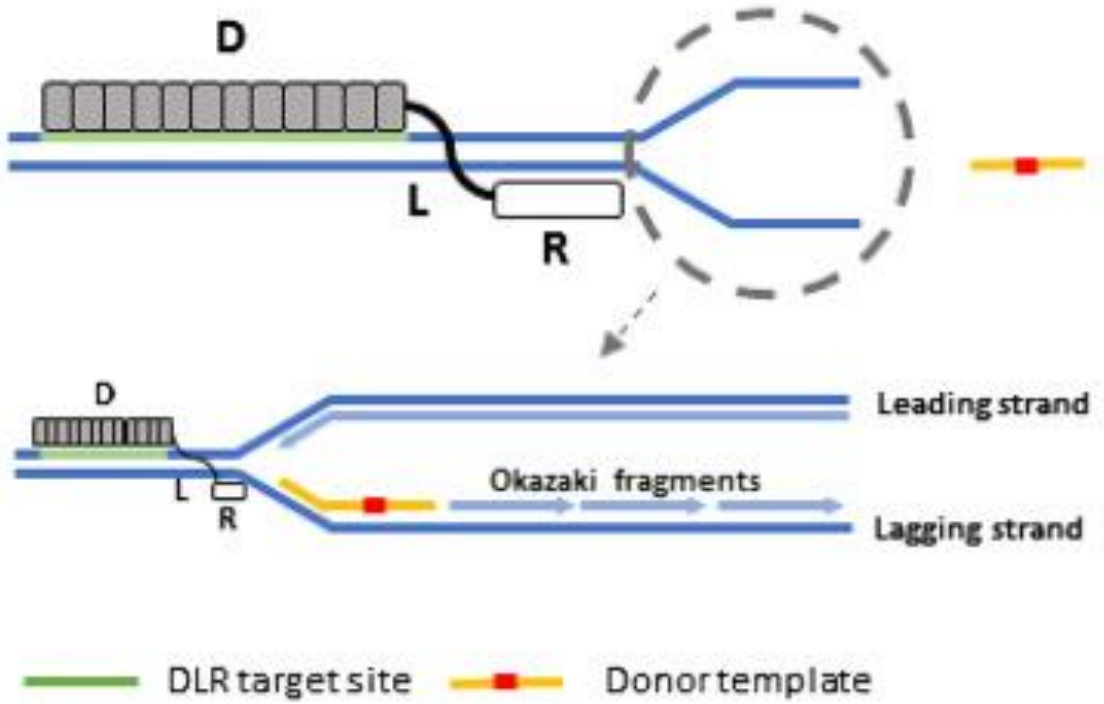
New Tools



Create a "landing pad" at a specific location. Old hat technology platform in plants; however, the novelty is using PRIME Editing to create the target site. Landing pads are safer and more reliable than double—strand breaks.

Yarnall, Matthew TN, et al. "Drag-and-drop genome insertion of large sequences without double-strand DNA cleavage using CRISPR-directed integrases." Nature Biotechnology (2022): 1-13.

New Tools



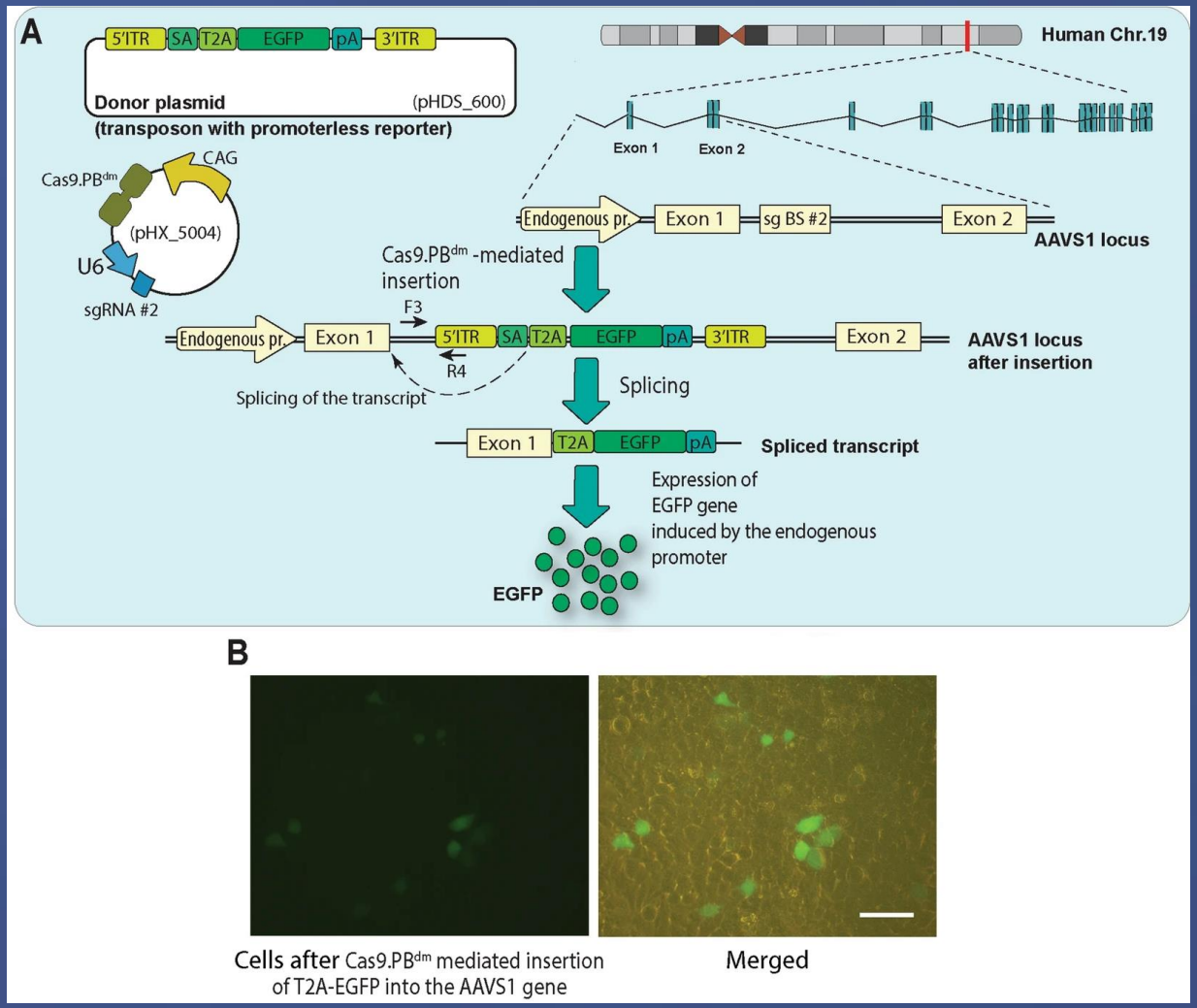
Cleavage-Free Human Genome Editing

Avoiding making any breaks (ss or ds) sneaking into DNA during replication

Downside, requires replication.

Kuang, Chenzhong, Yan Xiao, and Dirk Hondmann. "Cleavage-free human genome editing." Molecular Therapy 30.1 (2022): 268-282.

New Tools

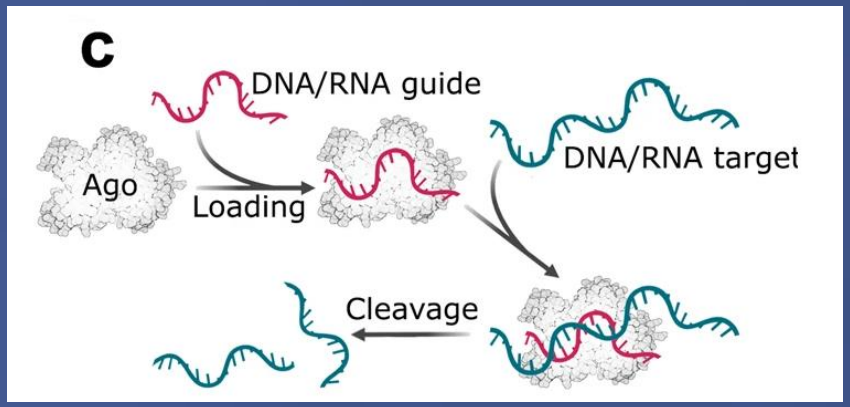


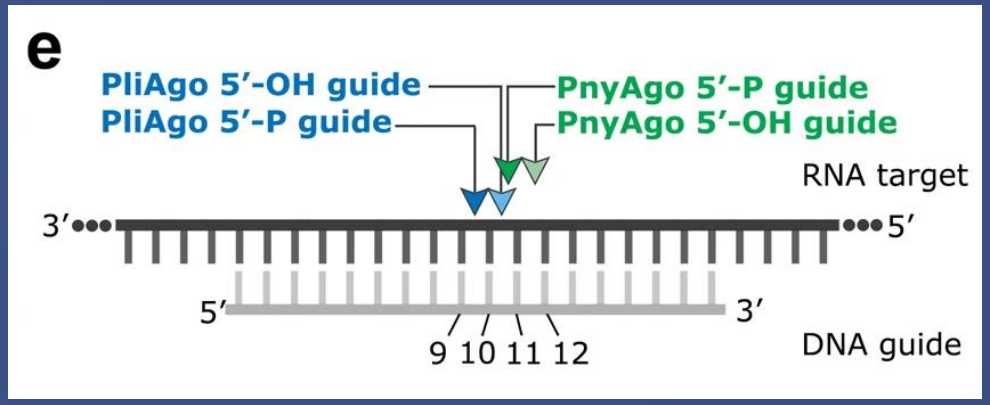
Again, using yet another kind of endonuclease activity to avoid a double-strand break

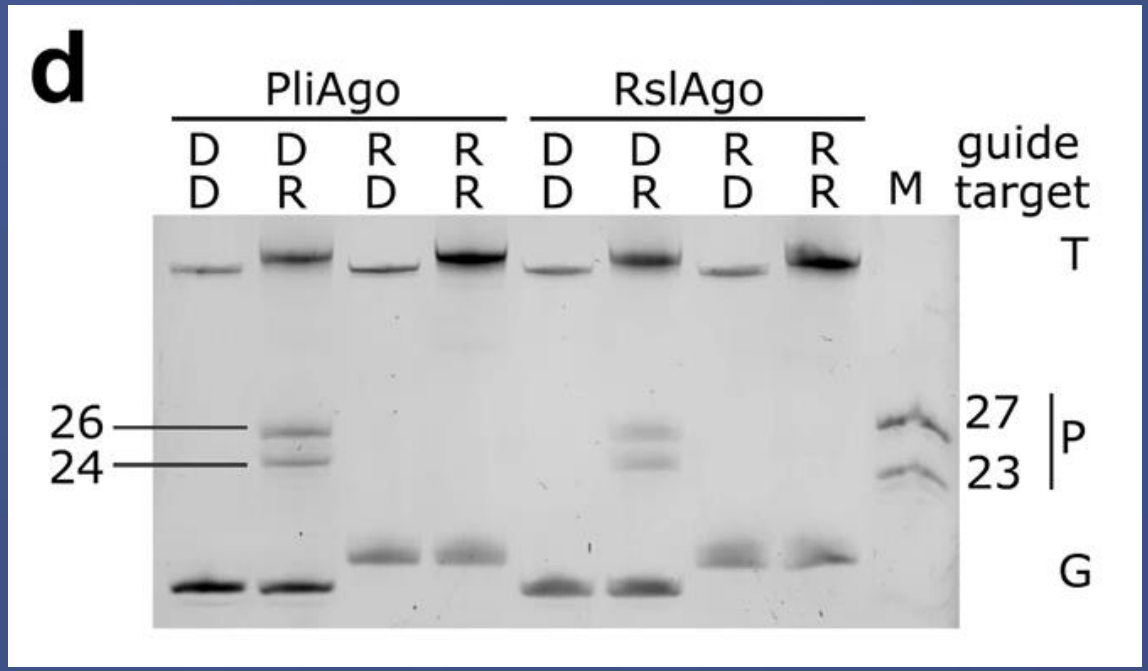
Also what I liked, the design strategy for changing genes

Rezazade Bazaz, Mahere, Mohammad M. Ghahramani Seno, and Hesam Dehghani. "Transposase-CRISPR mediated targeted integration (TransCRISTI) in the human genome." Scientific reports 12.1 (2022): 1-18.

New Tools





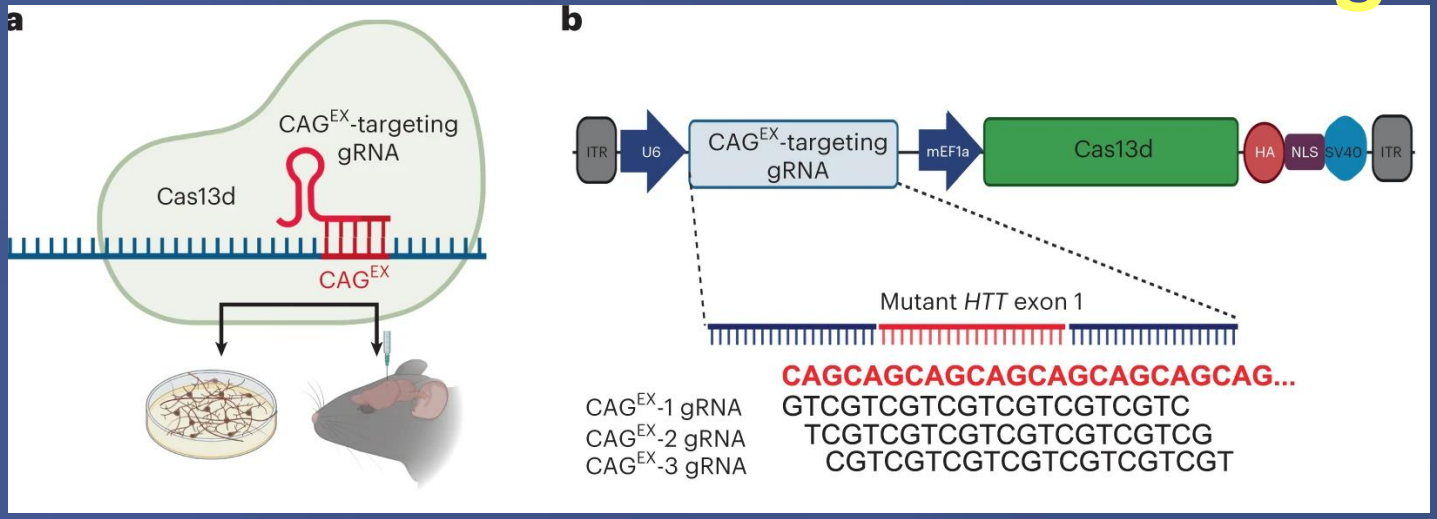


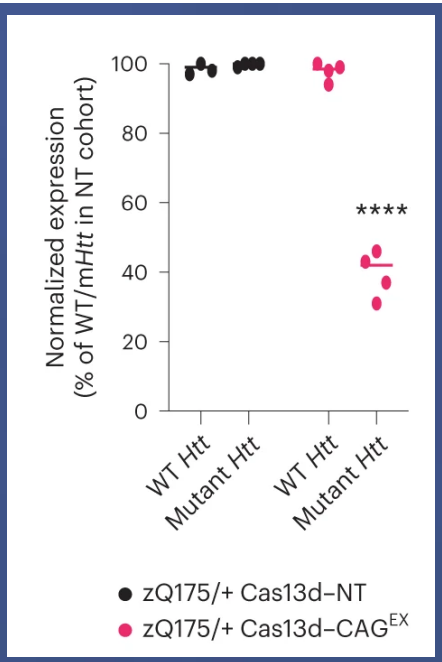
Another class of “editors” (prokaryotic Argonaute) can now also target RNA

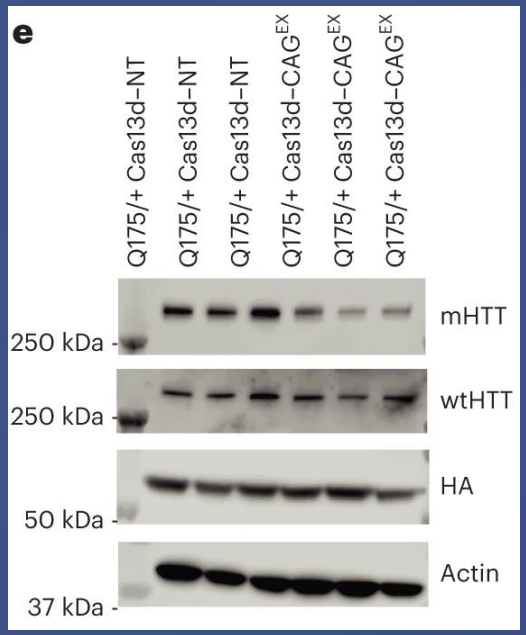
Another lesson here, if you look smartly enough into diverse genomes, you’ll find what you are looking for

Lisitskaya, Lidiya, et al. "Programmable RNA targeting by bacterial Argonaute nucleases with unconventional guide binding and cleavage specificity." Nature communications 13.1 (2022): 1-15.

Tech Advances – RNA targeting



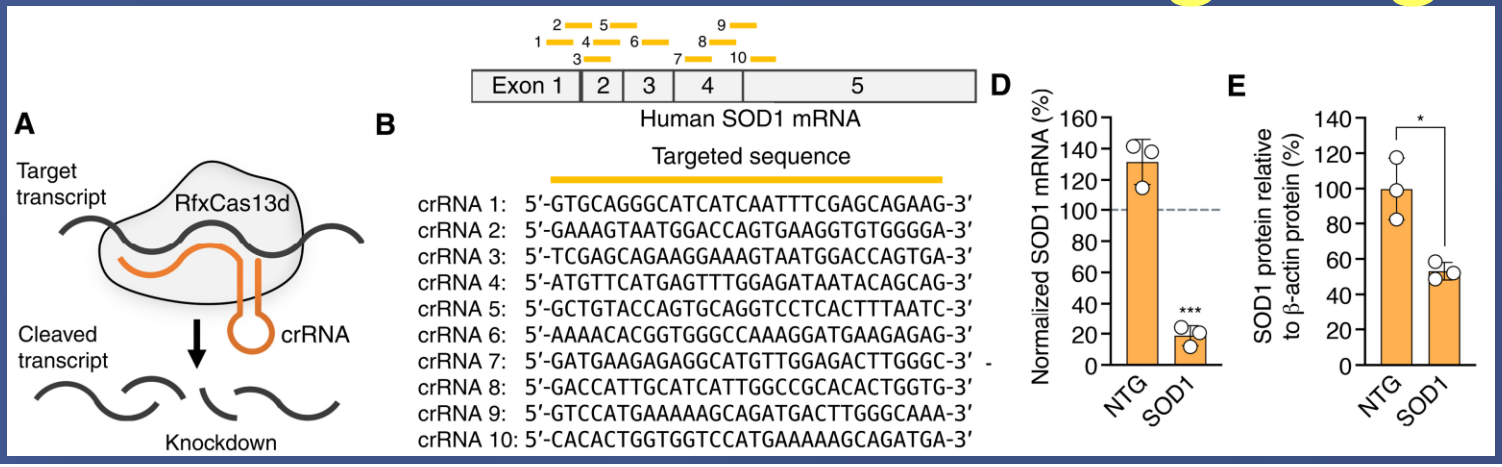


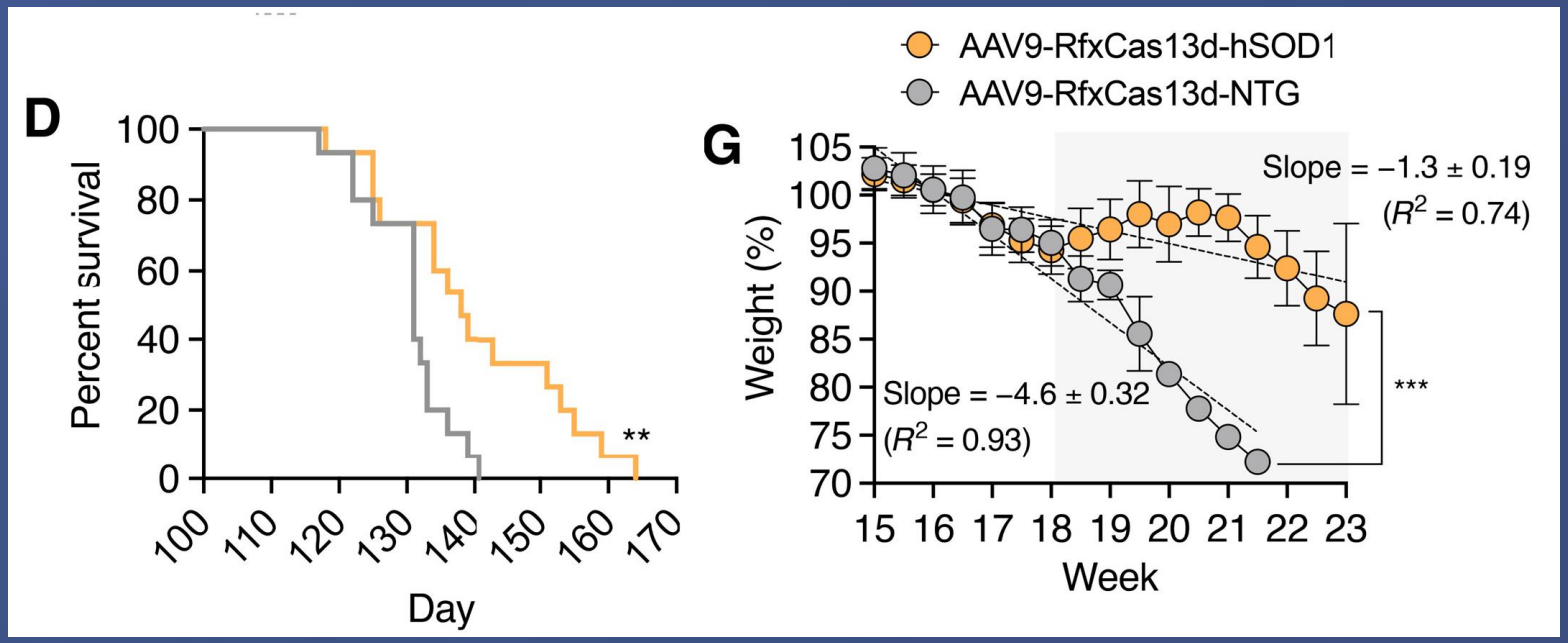


This is not technically novel, but highlighting because the effectiveness in animal models for certain diseases is compelling.

Morelli, Kathryn H., et al. "An RNA-targeting CRISPR–Cas13d system alleviates disease-related phenotypes in Huntington's disease models." Nature Neuroscience (2022): 1-12.

Tech Advances – RNA targeting

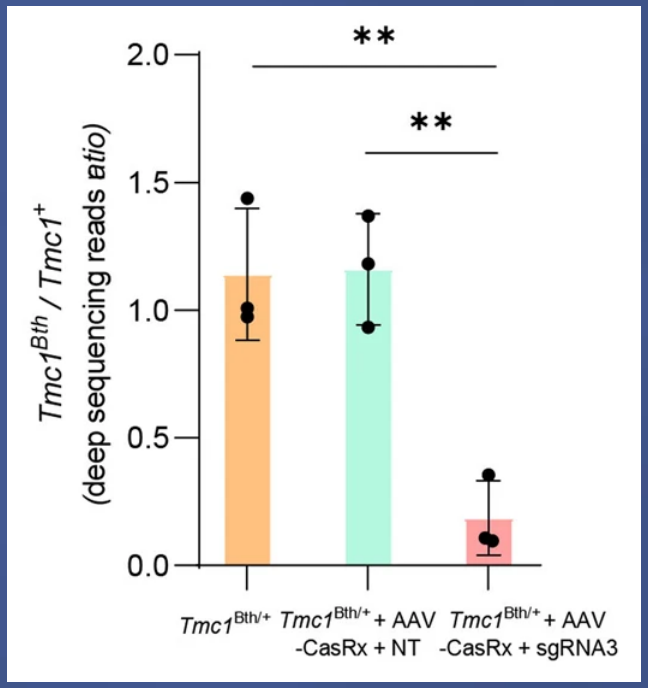


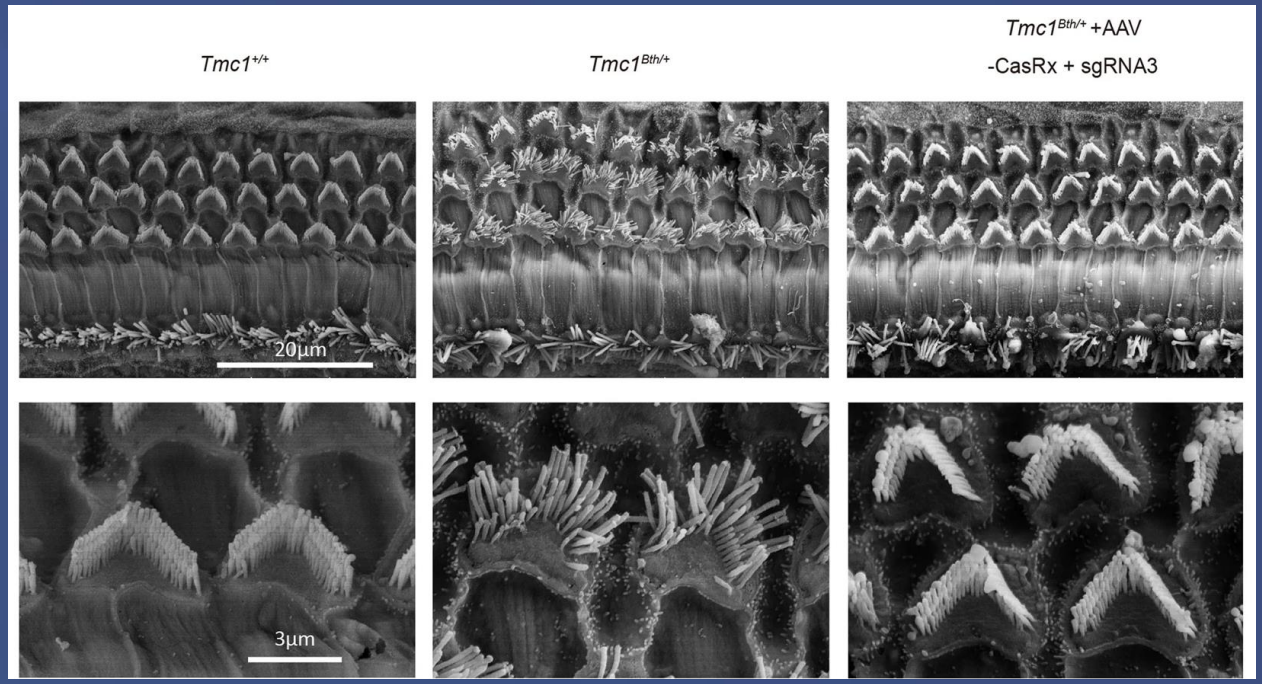


Remember – neurons are non dividing cells. Mutated SOD1 leads to ALS

Powell, Jackson E., et al. "Targeted gene silencing in the nervous system with CRISPR-Cas13." Science advances 8.3 (2022): eabk2485.

Tech Advances – RNA targeting

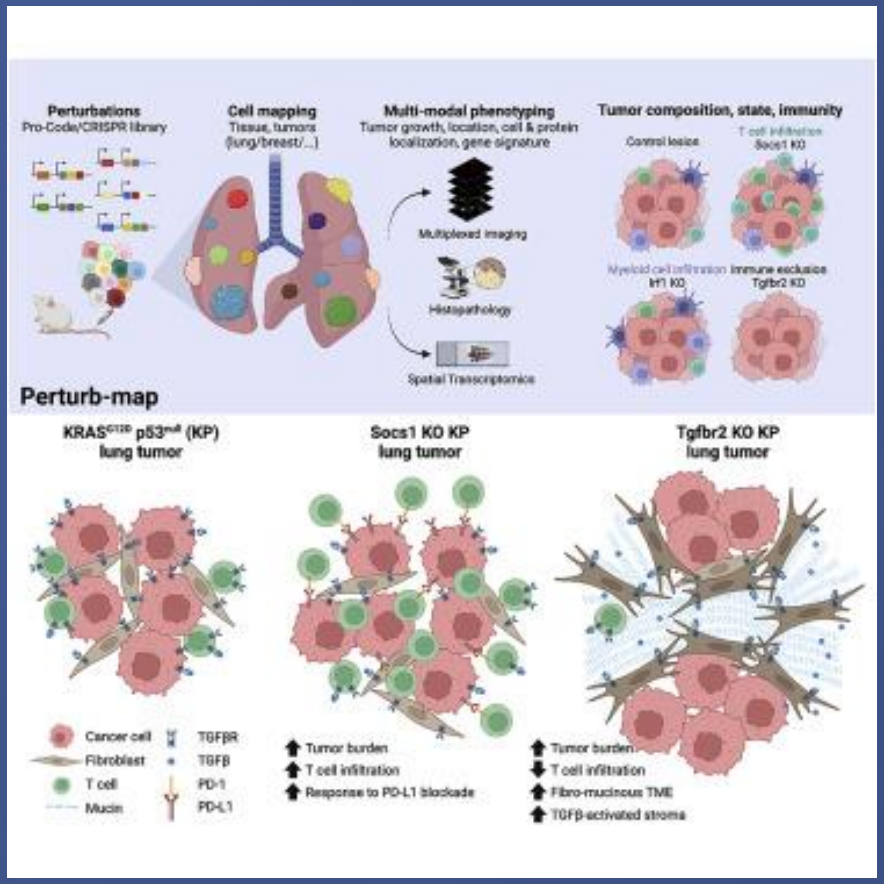




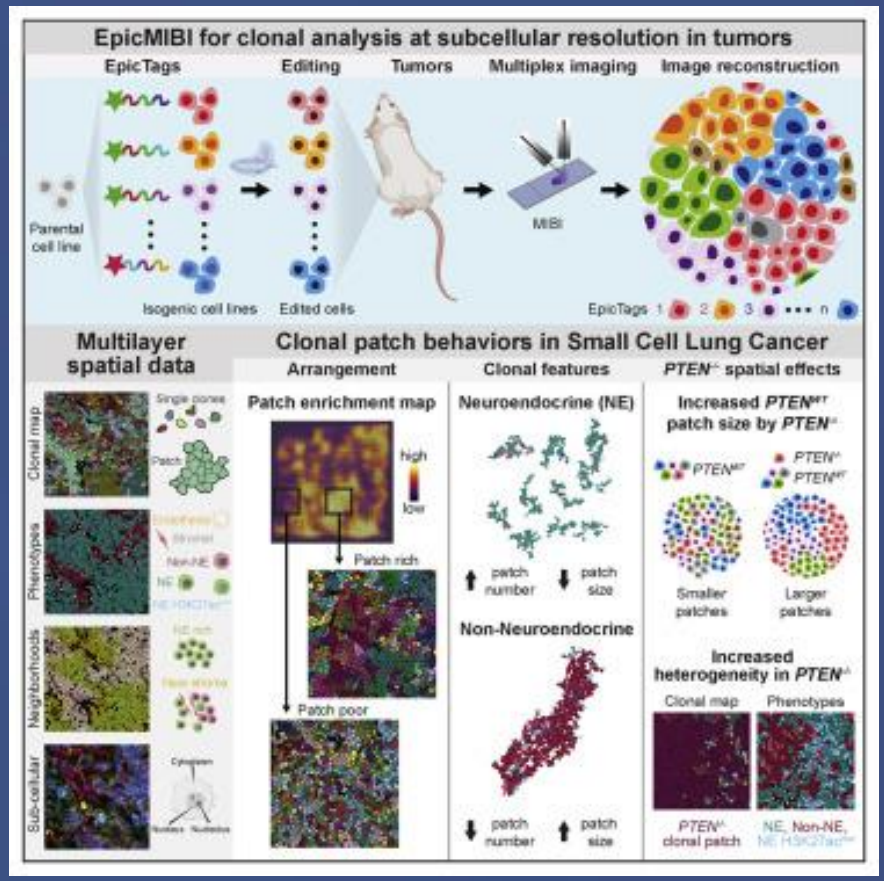
Effective enough via AAV delivery to fix complex structures, highlight autosomal dominant nature of disease targeting allele-specific version

Zheng, Ziwen, et al. "Preventing autosomal-dominant hearing loss in Bth mice with CRISPR/CasRx-based RNA editing." Signal transduction and targeted therapy 7.1 (2022): 1-13.

New Uses



Screen for extra-cellular function within tumor microenvironment

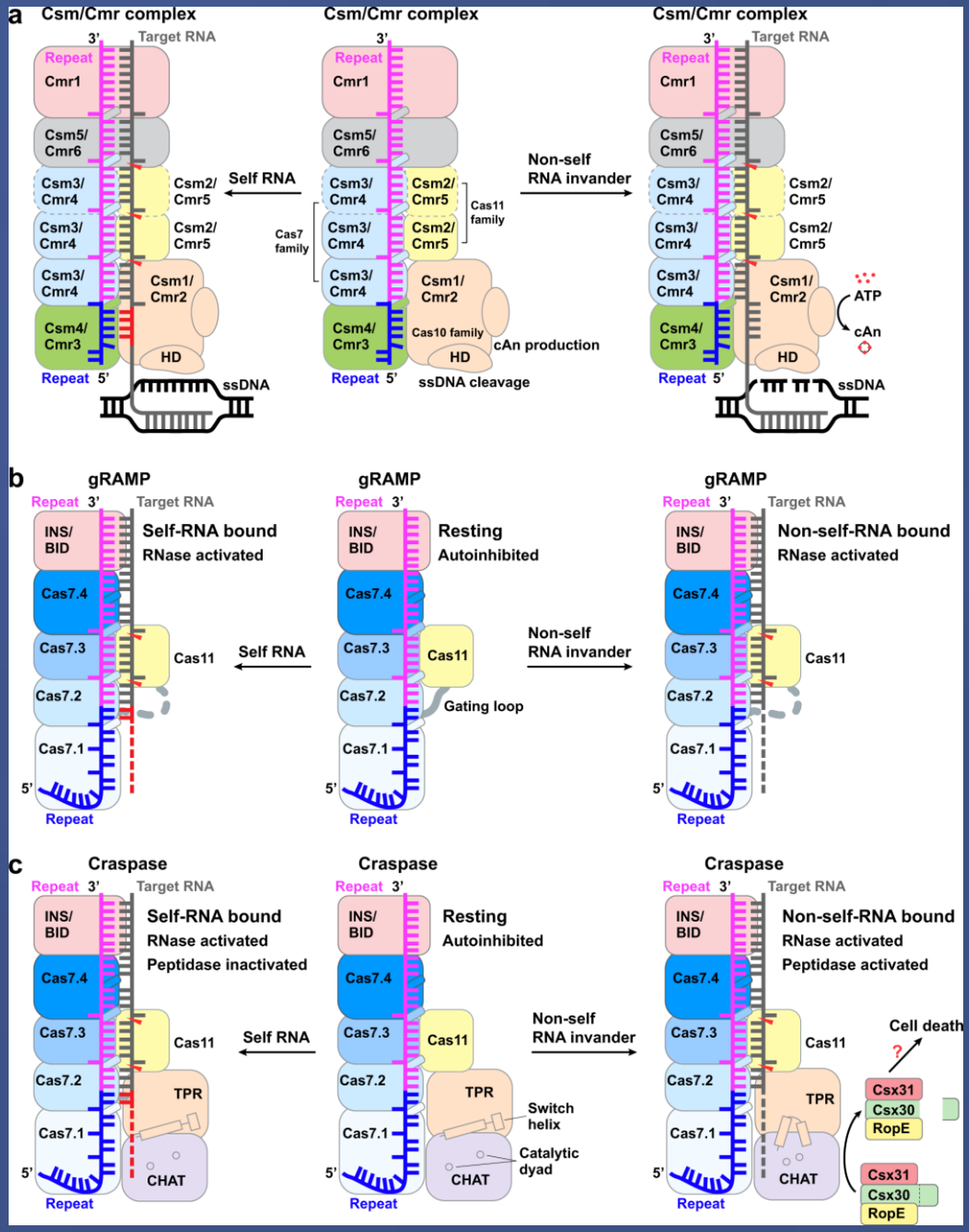


Visual tags

Dhainaut, Maxime, et al. "Spatial CRISPR genomics identifies regulators of the tumor microenvironment." Cell 185.7 (2022): 1223-1239.

Rovira-Clavé, Xavier, et al. "Spatial epitope barcoding reveals clonal tumor patch behaviors." Cancer Cell 40.11 (2022): 1423-1439.

New Uses



This looks complicated, but key function is ability to activate a PROTEASE associated with the complex

Type III-E

From targeting a specific RNA

Used in prokaryotes to "self-destruct" in the face of viral infection, caspase-like

PROTEASE targets are host proteins

Hu, Chunyi, et al. "Craspase is a CRISPR RNA-guided, RNA-activated protease." Science 377.6612 (2022): 1278-1285.

Policy News

PRLR-SLICK cattle contain a mutation in the prolactin receptor (PRLR) gene that results in a shortened prolactin receptor protein and a ‘slick’ hair phenotype that allows the animals to better withstand tropical heat.

Based on the safety of consumption of meat from conventional breeds with the same mutation, the FDA finds no reason to regulate these CRISPR cattle. Acceligen provided the FDA with whole-genome sequencing data. In the two founder cattle, the FDA found several unintended variations, none of which they believe affect the health and safety of the cattle or its products, being mainly small insertions and deletions in intergenic regions.

Harrison, Charlotte. "CRISPR beef cattle get FDA green light." (2022): 448-448.

Policy News

Kenya's National Biosafety Authority (NBA) has published Genome Editing Guidelines, marking an important step towards the development of a genome editing regulatory framework in the country. After Nigeria, Kenya becomes the second African country to publish such guidelines.

The published guidelines provide clarity on which genome-edited organisms and/or derived products will be regulated under Kenya's Biosafety Act, and which products are regulated as conventional varieties or breeds.

"Kenya joins Nigeria in Africa in approving guidelines for CRISPR and other gene editing techniques"
https://geneticliteracyproject.org/2022/03/18/kenya-joins-nigeria-as-african-countries-approving-guidelines-for-crispr-and-other-gene-editing-techniques

Policy News

The Bill completed its Commons stages unamended with the Report Stage and Third Reading held on 31 October 2022.

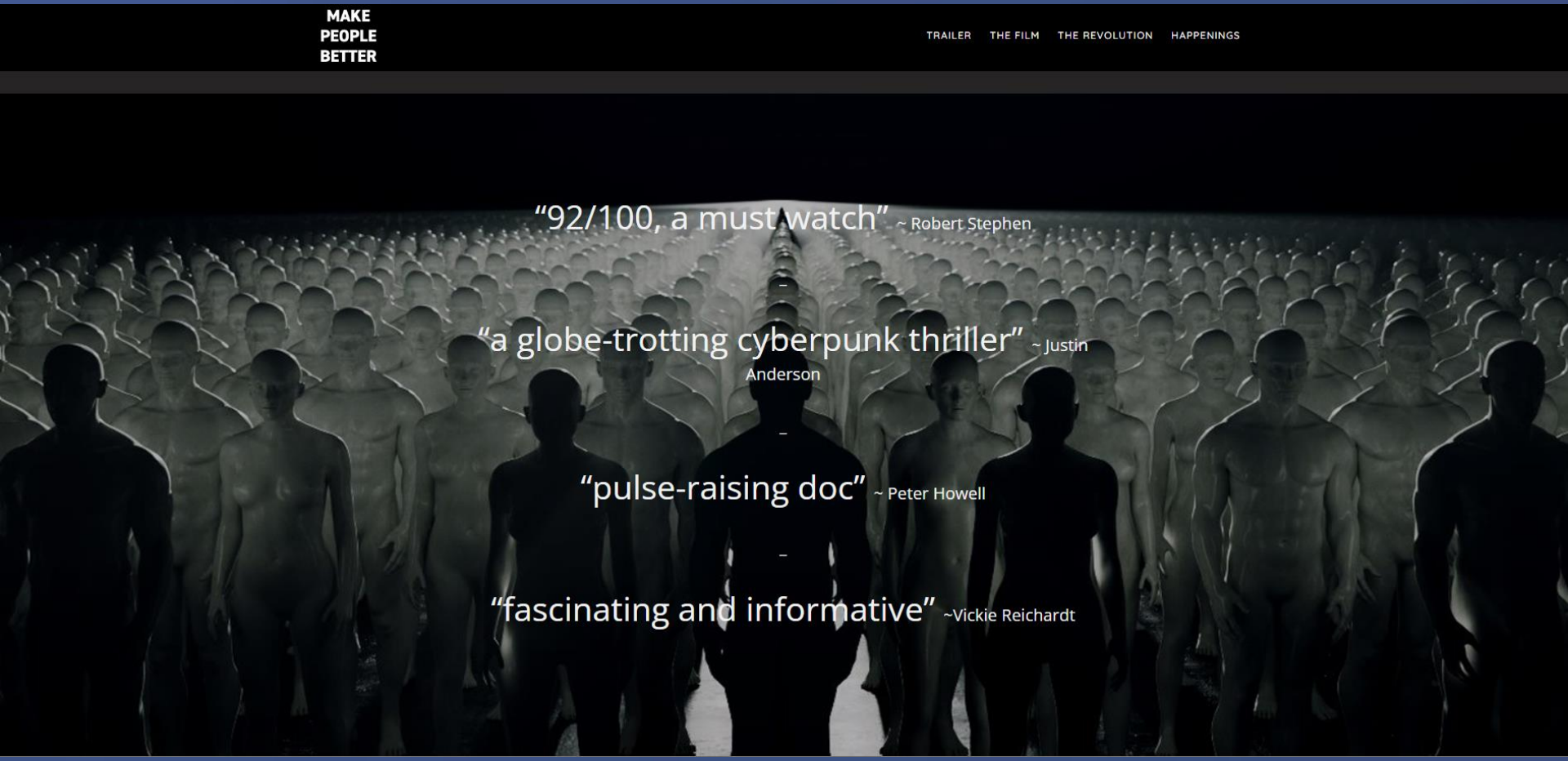
What would the Bill do?
The May 2022 Queen's Speech said the Bill's aims are to "encourage agricultural and scientific innovation" in the UK" and that "legislation will unlock the potential of new technologies to promote sustainable and efficient farming and food production."

The Bill applies to precision bred plants and vertebrate animals (excluding humans), meaning they are gene edited, and would remove them from the regulatory system for genetically modified organisms (GMOs).

Genetic Technology (Precision Breeding) Bill 2022-23
https://commonslibrary.parliament.uk/research-briefings/cbp-9557

https://makepeoplebetterfilm.com/

In the media



When the Chinese scientist He Jiankui announced in 2018 that he had successfully taken human embryos with genetically edited DNA and implanted them in a woman's uterus, it sparked international controversy among scientists and stoked deep-seated fears of normalizing "designer babies," which would allow the wealthy to buy the ability to choose the genetic characteristics of their offspring. In the documentary "Make People Better," the director Cody Sheehy dives into this complex story of genomic discovery, biomedical ethics and the covert dealings of the Chinese government.
https://www.nytimes.com/2022/12/14/movies/make-people-better-review.html

2022 CRISPR Year in Review

A very exciting year demonstrating

--use in the markets close to consumers/patients

--continued variety of tools and specialized applications

--more regulatory adoption in skeptical markets