Tools and applications of gene editing

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How could we edit DNA?

https://www.5sensesll.com/

Where do the tools come from?

"There's CRISPR in your yogurt"

http://www.eatingwell.com/

Bacteria armed with natural immune system – "CRISPR" are added to the starter culture of yogurt to remove virus (phage) contamination that affects production.

https://www.the-scientist.com/

https://www.pinclipart.com; https://disqus.com/

Can CRISPR do gene editing in other organisms too?

Development, 2014

Some examples

Agricultural biotechnology

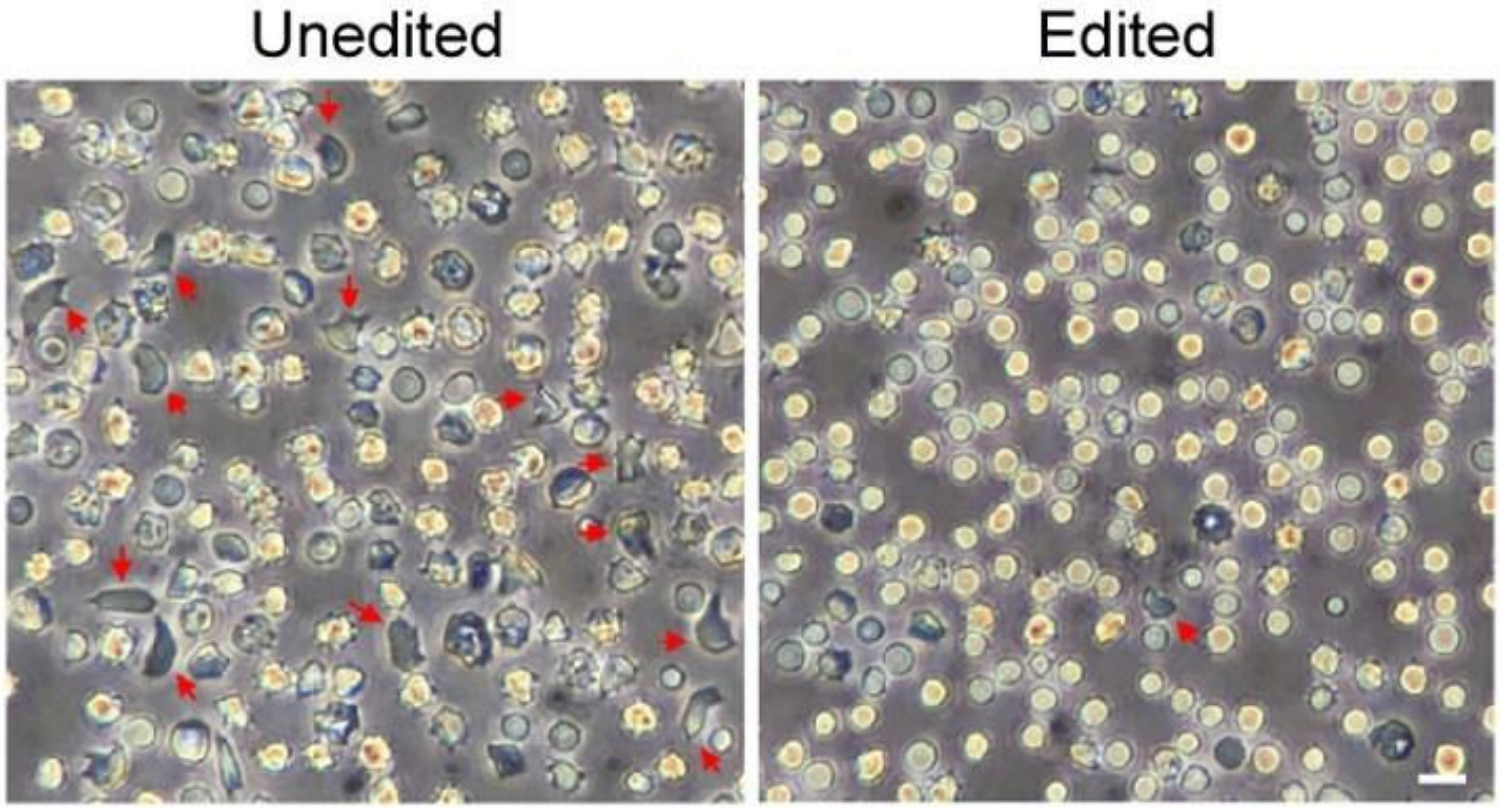
Development of new generations of genetically modified plants (e.g. a better drought-resistant plant, eliminate the irritant or allergenic properties of a plant, etc.)

Understand behavior

Advancing research tools for exploring gene functions in complex systems. For example, humans/mice with mutations in MC4R gene lack the satiation that normally occurs with eating, which lead to obesity and hyperinsulinemia.

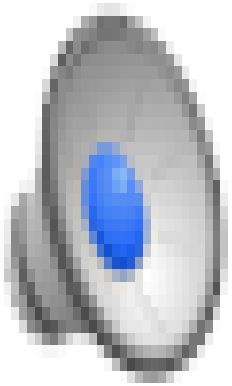
Gene therapy

Introduce an anti-sickling gene into the HSC to capitalize on the self-renewing potential of stem cells and create a continual source of healthy red blood cells that do not sickle. The gene therapy technique for sickle cell disease is in clinical trials.

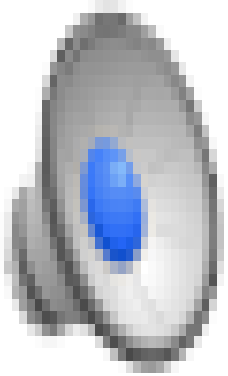


Nature Medicine, 2019

Wild type



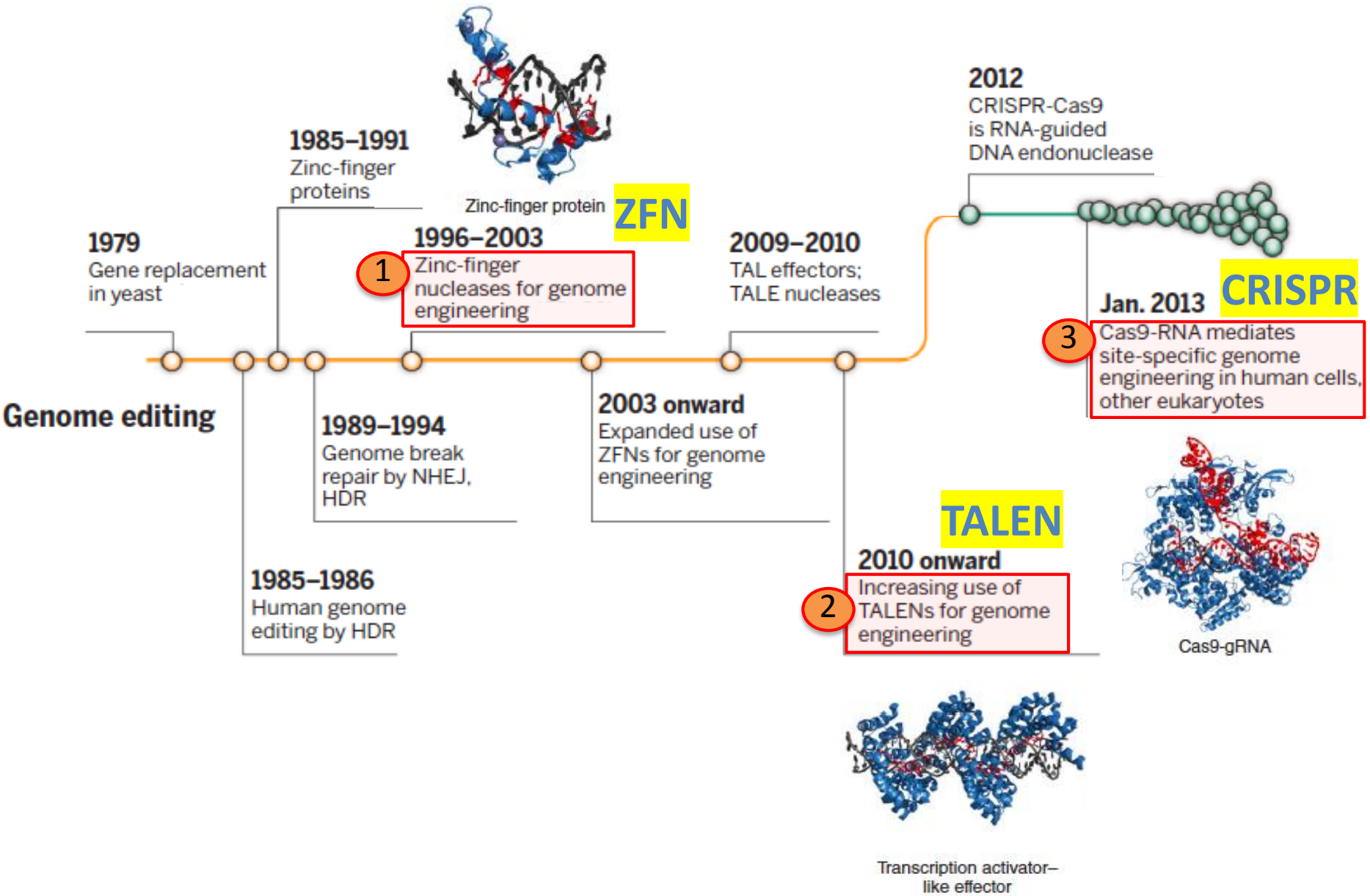
Mutant



While homozygous knockout offspring were physiologically indistinguishable from wild-type litter-mates, they showed specific domesticated behaviours: hypoactivity in the dark phase and a decline in the avoidance of a human hand. These phenotypes were consistent over subsequent generations. Our findings support the empirical hypothesis that *nonagouti* is a domestication-linked gene, the loss of which might repress aggressive behaviour.

Scientific Reports, 2017

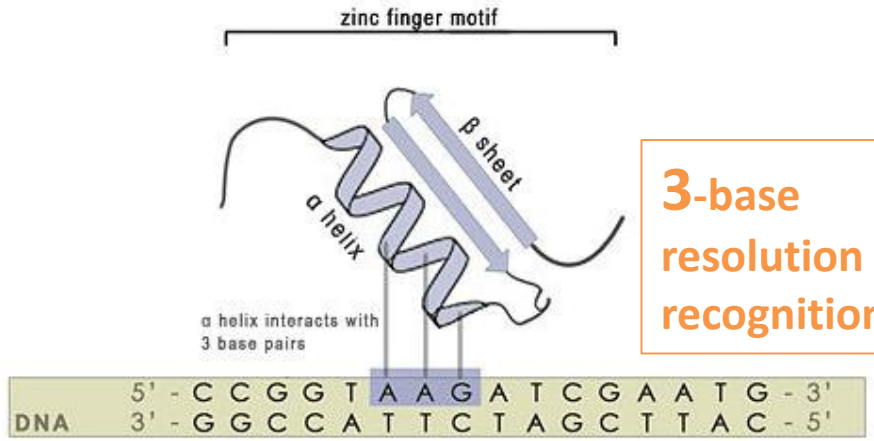
Programmable tools for gene editing



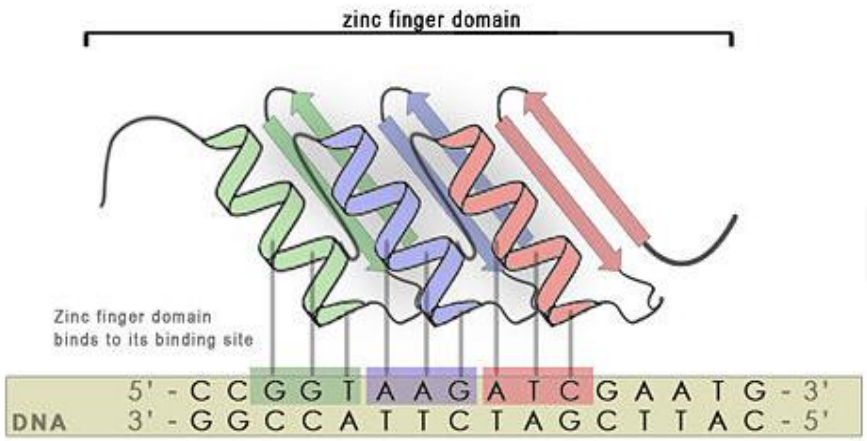
How to program?

010100101001111110	TCTCACTCGCGACTCGG
101001011001111000	ACGTCTCGCTTCGCCAA
000111010101001001 0	CGTCTAGGCCACTCTCG
110010101011001110 0	ACACTAAGAACGCGTCT
110000111001100001 1	GCTACTCCGCGCGTCTG
110000011111010101 0	TCGCGCTCGAAACTCGG

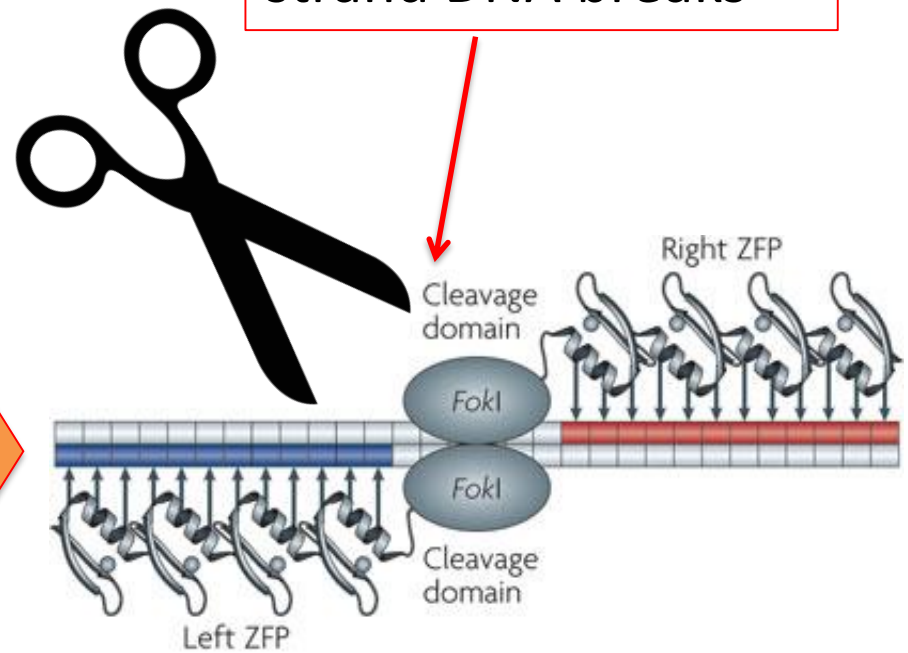
Zinc finger nuclease (ZFN)



3-base resolution of recognition

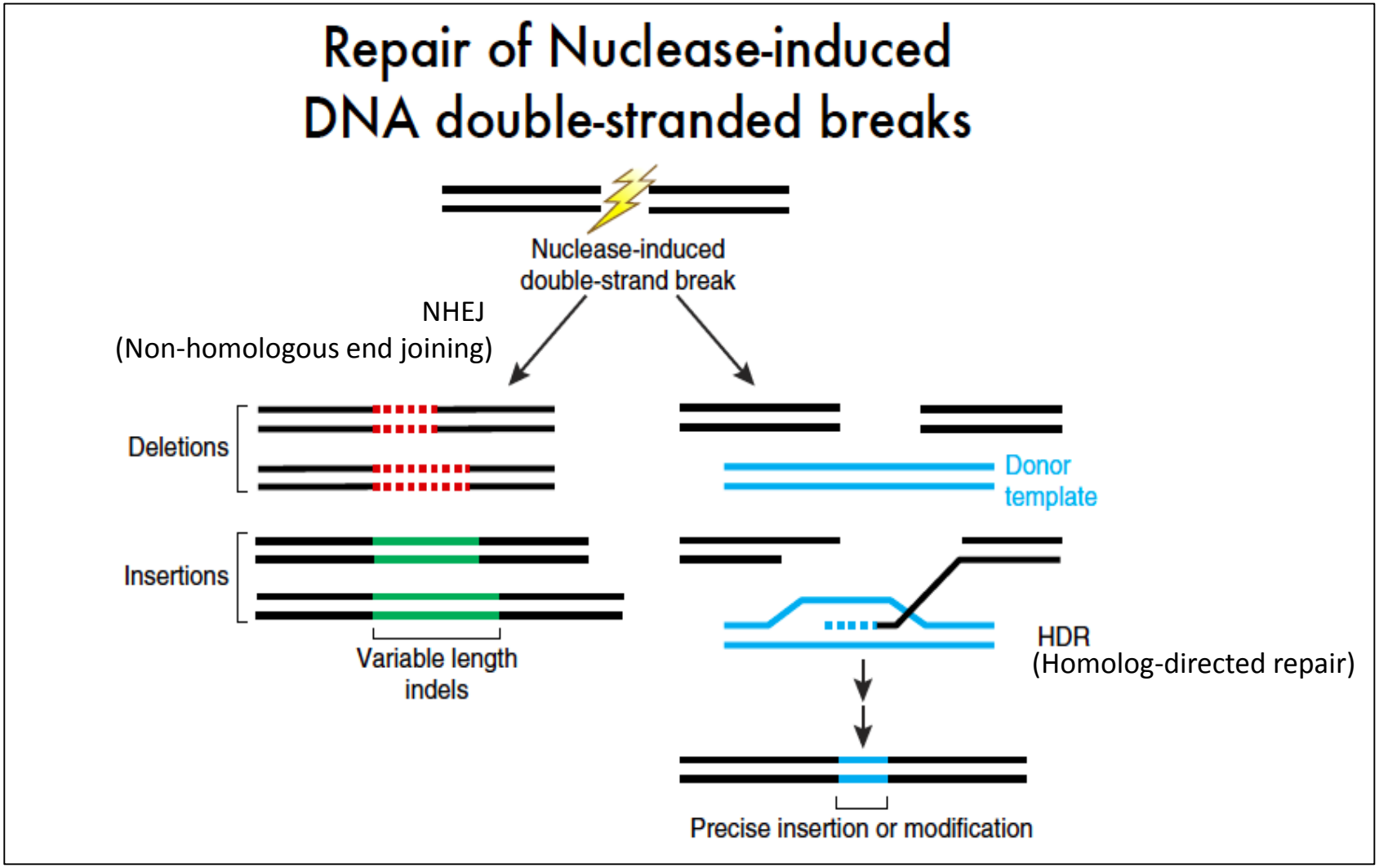


Generate double-strand DNA breaks



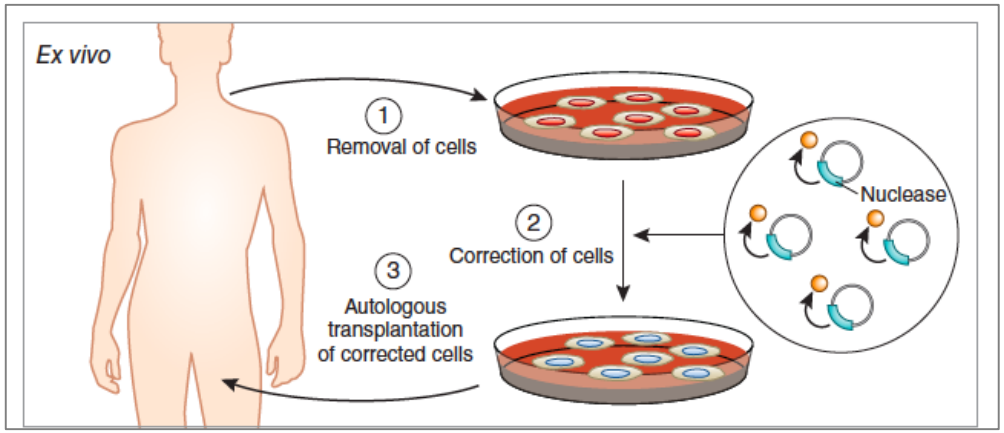
Edited from Nat. Rev. Genet., 2010; http://2010.igem.org/

Mechanism of gene editing



Nat. Biotech., 2014

ZFN tackles HIV in clinical test



- **C-C chemokine receptor type 5** (**CCR5**) is a protein expressed on immune T cell surface, which is a major co-receptor for human immunodeficiency virus (HIV)

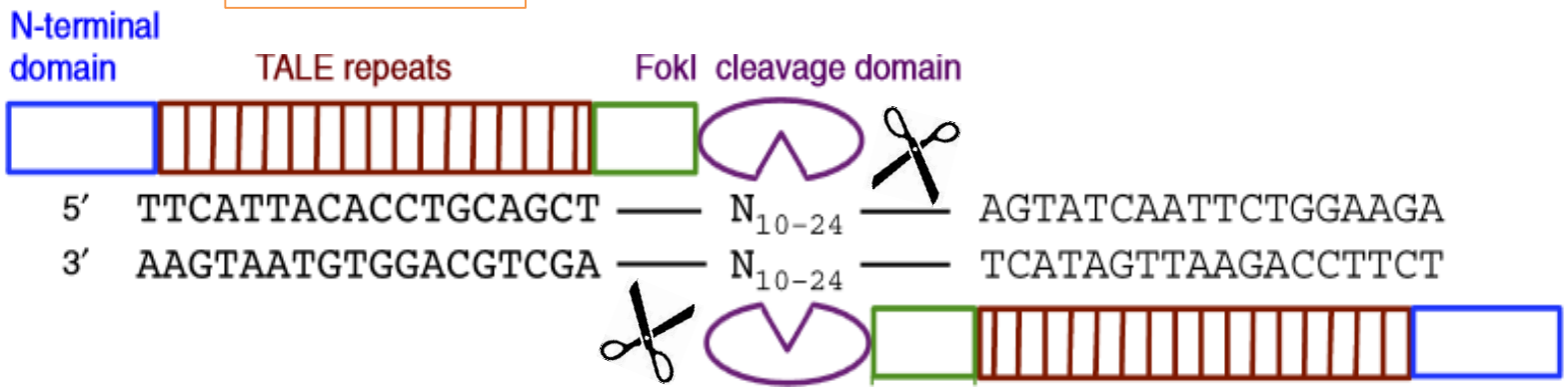
- a few individuals carry a mutation known as CCR5-delta32, protecting them against these strains of HIV.

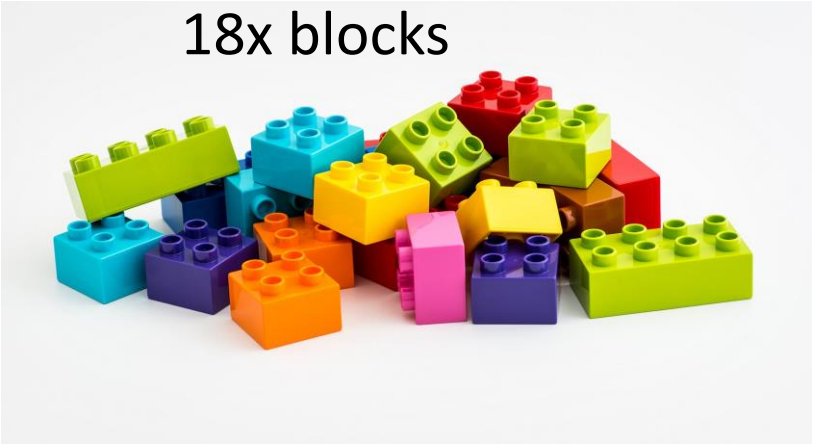
A clinical trial has shown that ZFN technique can be safe and effective in humans to target and destroy *CCR5* gene in T cells, increasing their resistance to HIV. The researchers transfused back all of the altered cells into the 12 participants. After treatment, their HIV levels rebounded more slowly, and their T-cell levels remained high for weeks in their blood, suggesting that the virus was less capable of destroying them.

NEJM., 2014; Nat. Med., 2015

TALE nuclease (TALEN)

1-base resolution of recognition



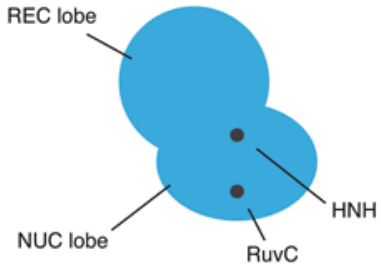


Nat. Methods., 2014

CRISPR nuclease

SCISSORS

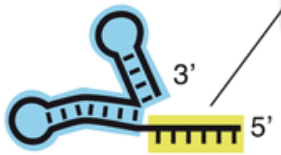
nuclease
Cas9



+

GUIDE

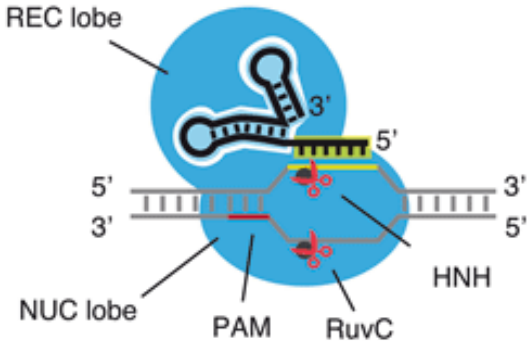
target-specific
sgRNA



programmable
sequence (20nt)

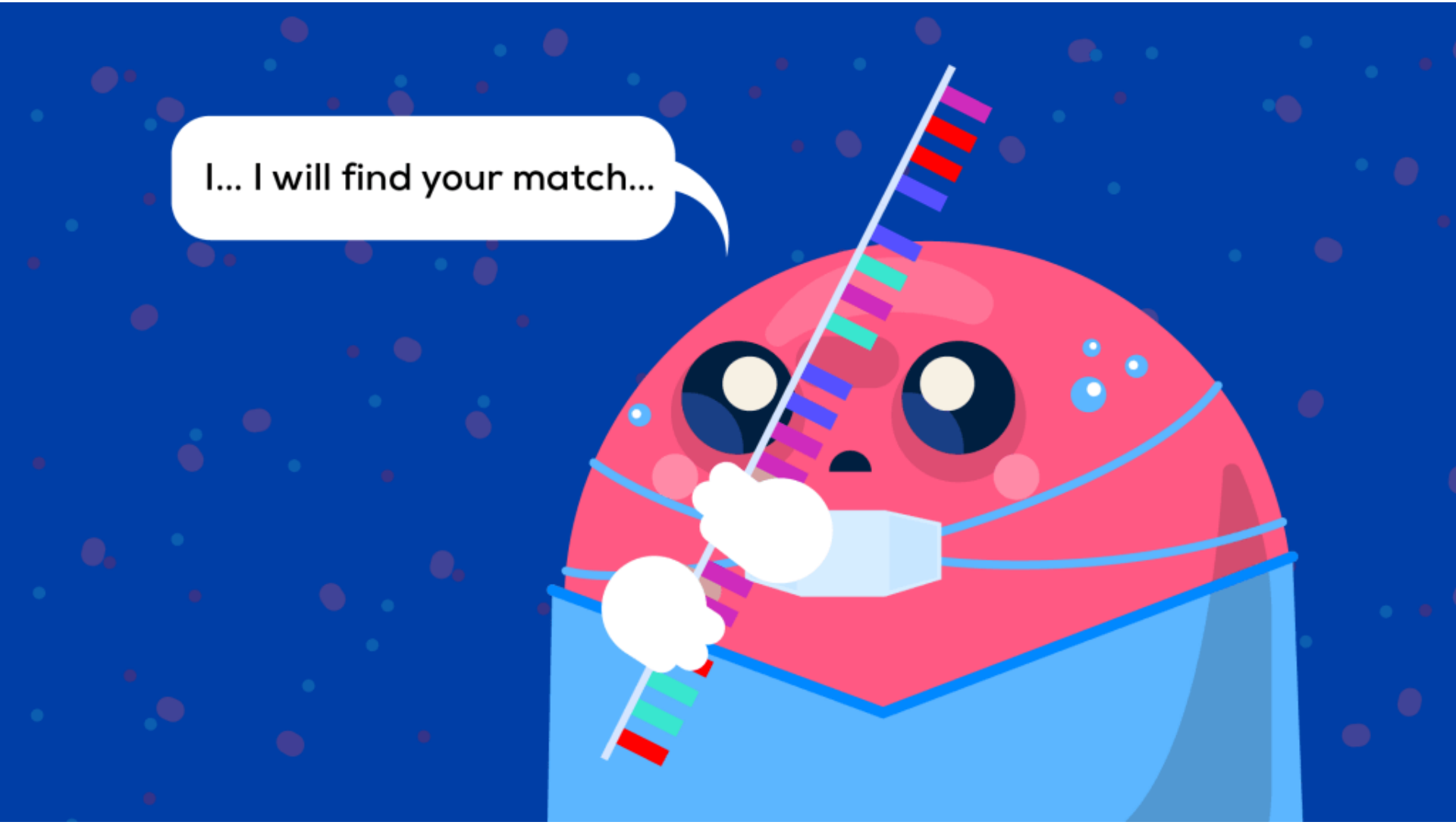
e.g.
GAGCCGAGACGAGCGAGACC

A single protein Cas9 guided by a single RNA



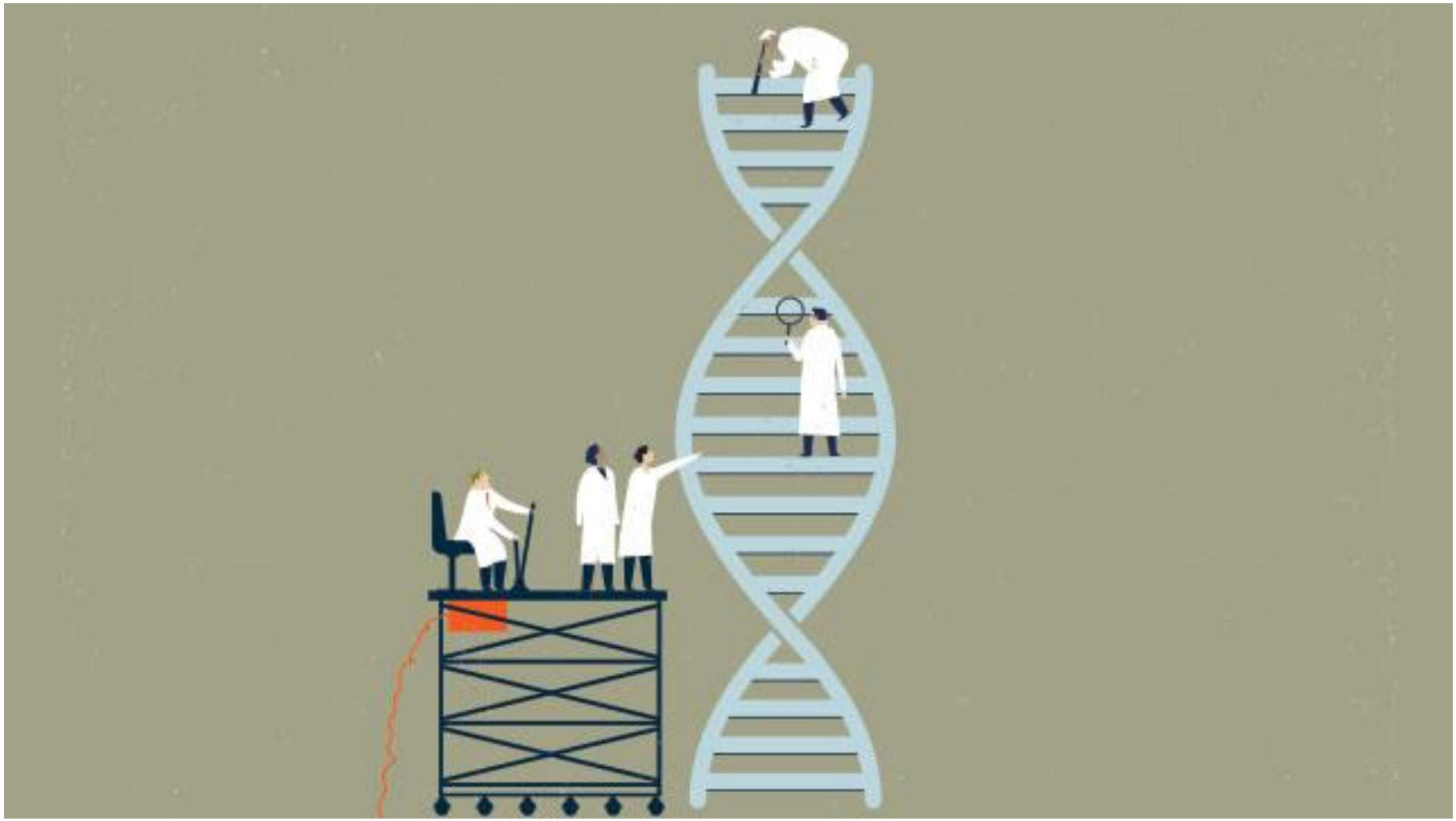
Cut the target DNA and repaired by NHEJ or HDR

F1000Res, 2017



https://disqus.com/

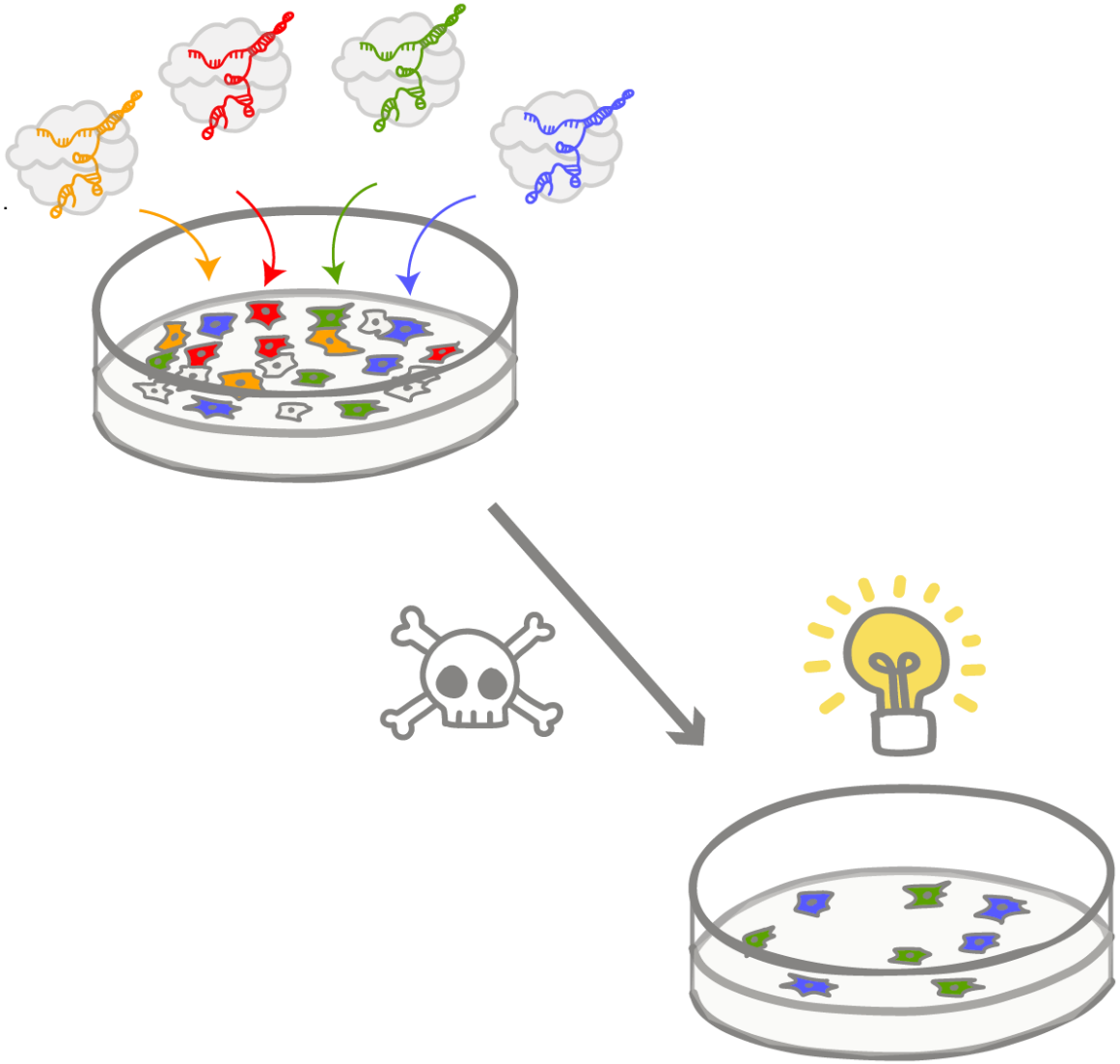
What will you do if you can access the genome using CRISPR technology?



https://www.the-scientist.com/lab-tools/new-methods-to-detect-crispr-off-target-mutations-30013

CRISPR screen

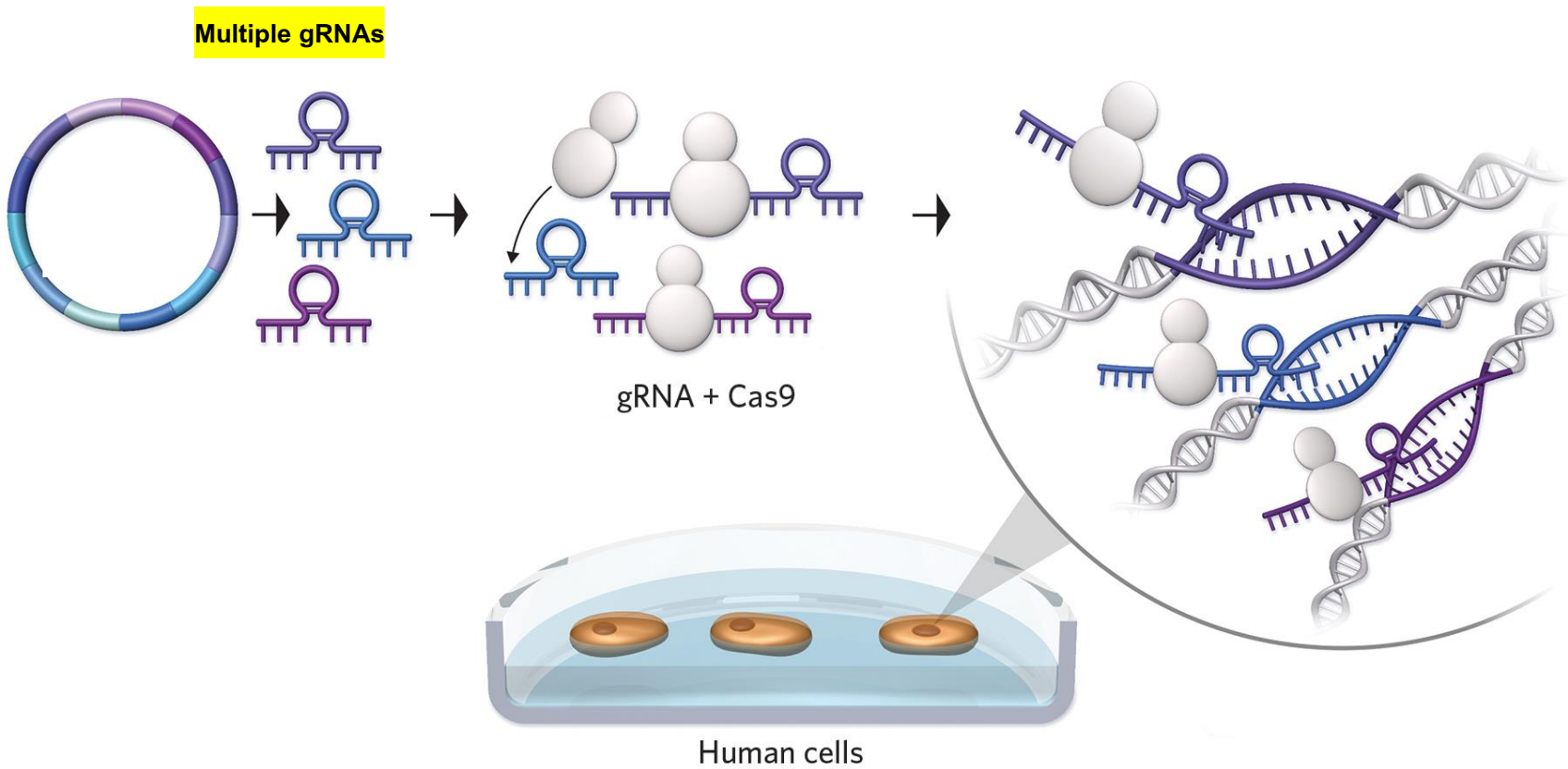
Instead of checking one gene at a time…



Whole-genome screening

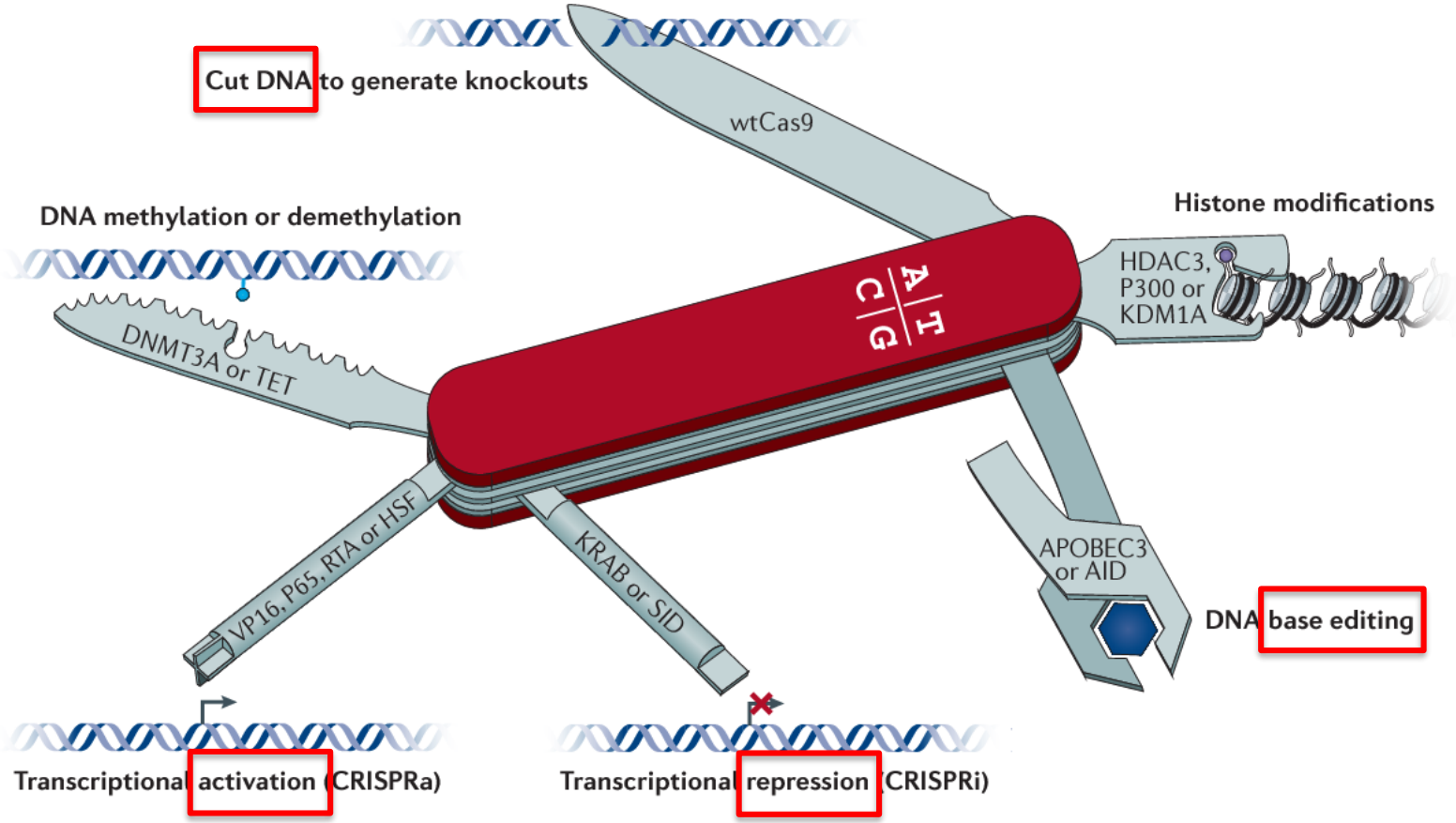
https://innovativegenomics.org/resources/educational-materials/glossary/crispr-screening/

Multiplexed CRISPR screen



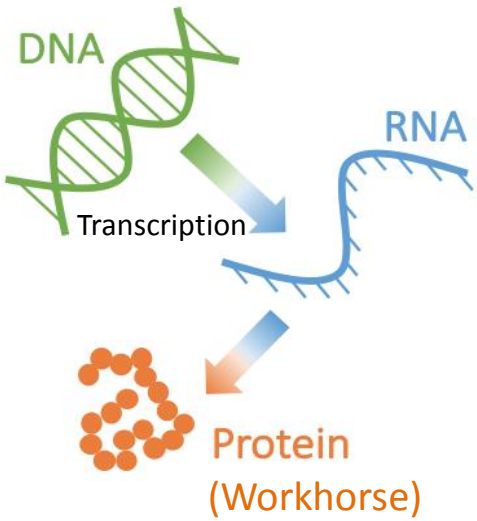
https://www.the-scientist.com/

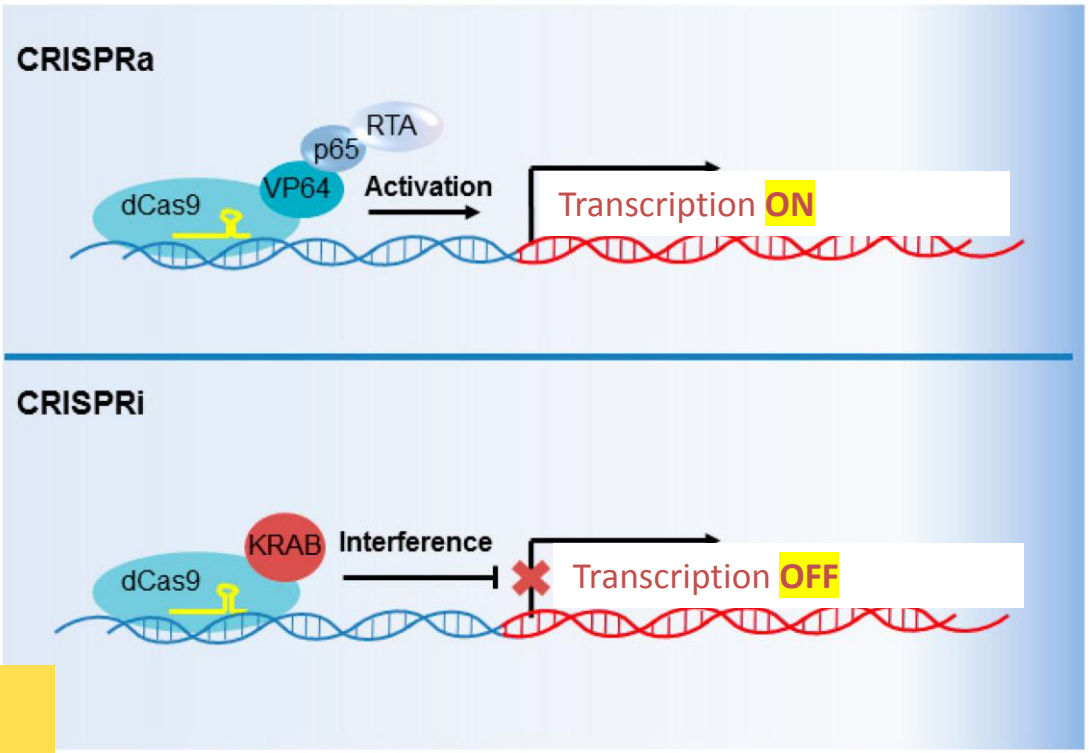
Using CRISPR to do more than cut

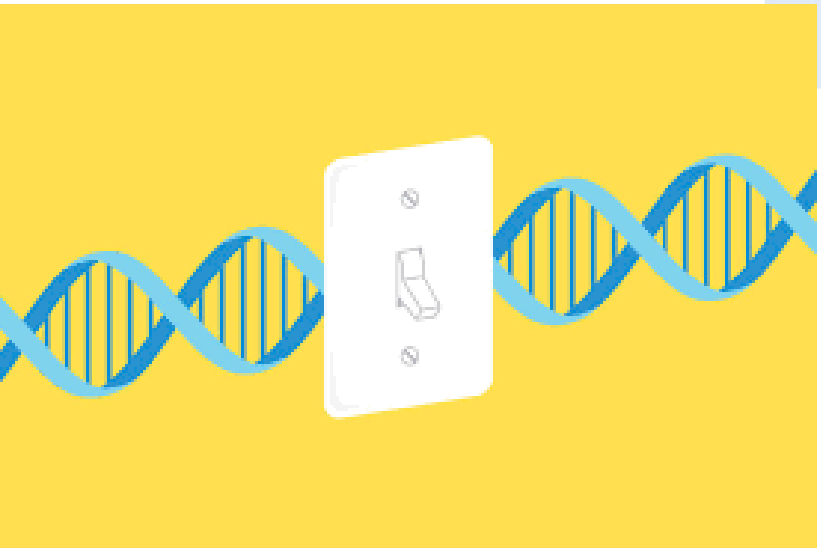


https://www.semanticscholar.org/

CRISPR for activating/repressing transcription





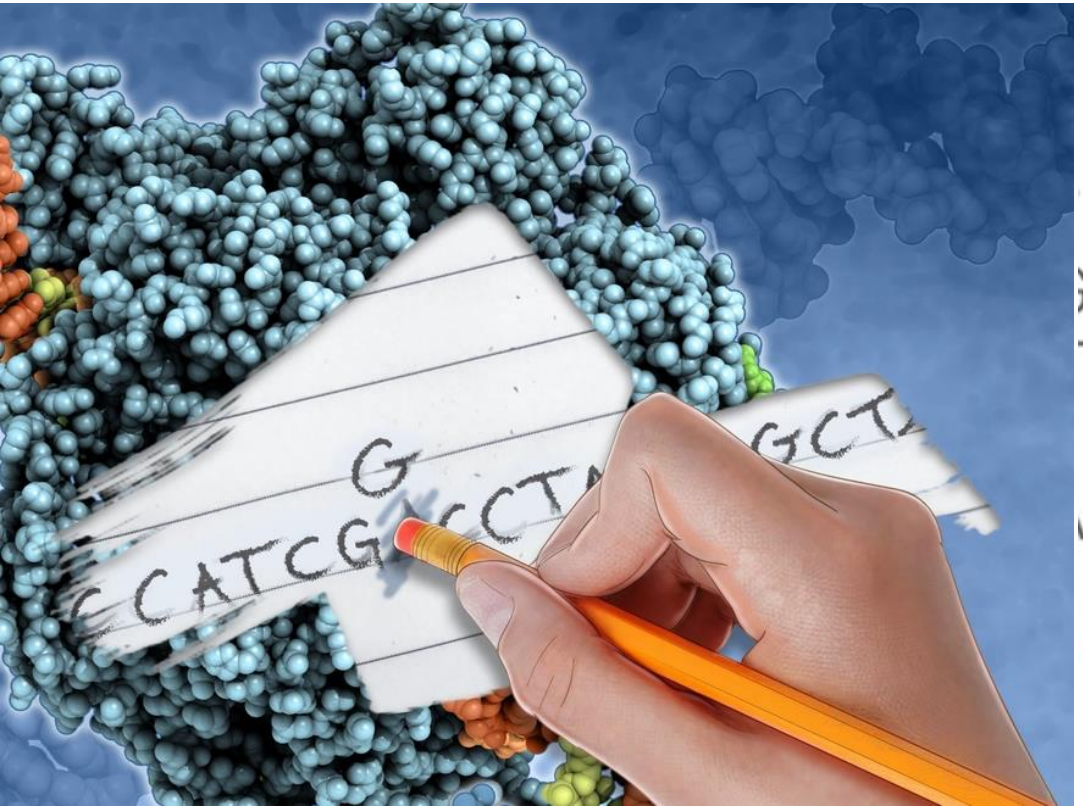


https://medicine.wustl.edu/

CRISPR for base editing

"Over half of genetic errors driving disease are the result of point mutations – a single-letter change in the billions of As, Ts, Cs, and Gs forming the genome. Changing that one base "back to normal" in enough cells may help us prevent, modify, and even cure serious genetic diseases."

https://beamtx.com

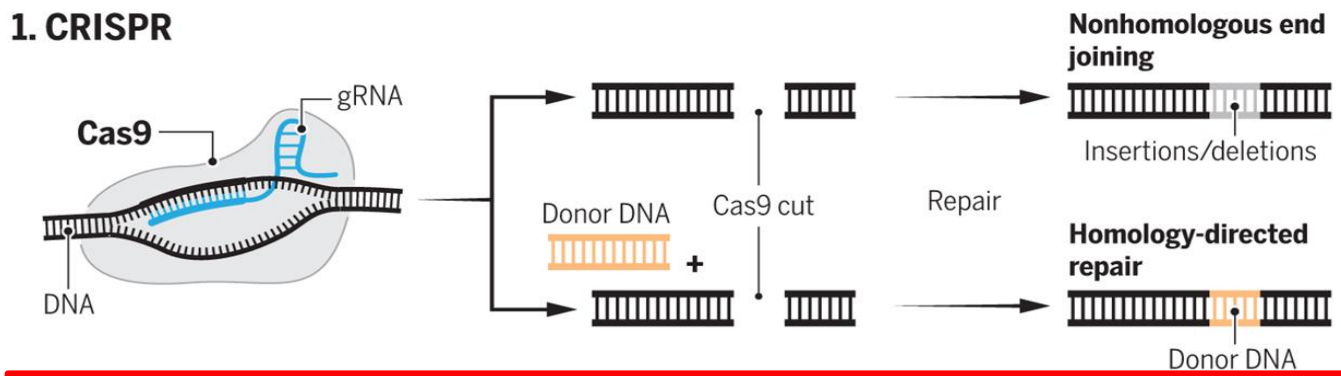


https://news.harvard.edu/gazette/; https://www.fiercebiotech.com/biotech/

How does base editor work?

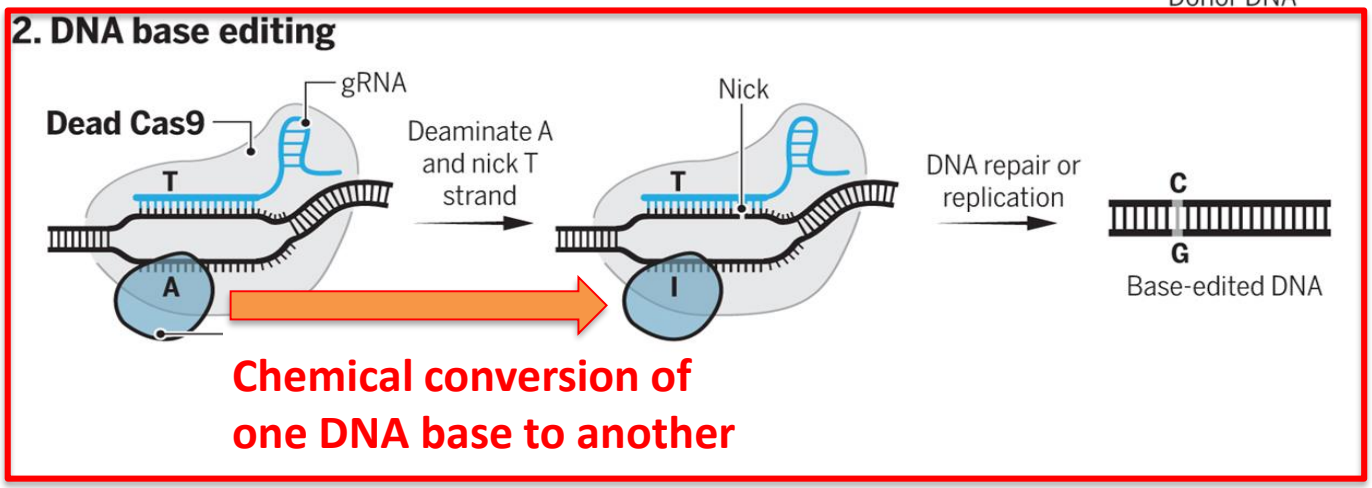
Getting to the point of mutations

Base editors borrow from CRISPR's components—guide RNAs (gRNAs) and Cas9 or other nucleases—but don't cut the double helix and instead chemically alter single bases with deaminase enzymes



Semi-random editing

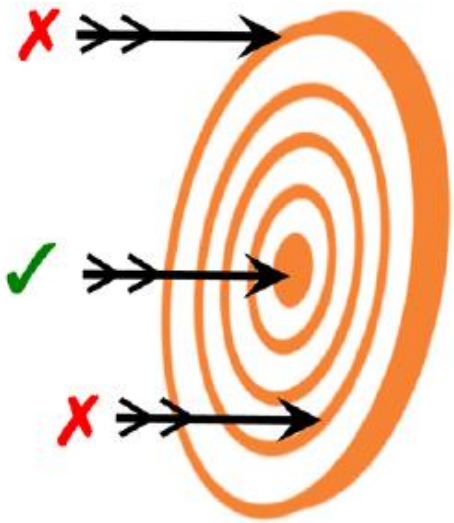
Low efficiency (~1-5%)



~5-50% efficiency

https://science.sciencemag.org/

Is gene editing ACCURATE?

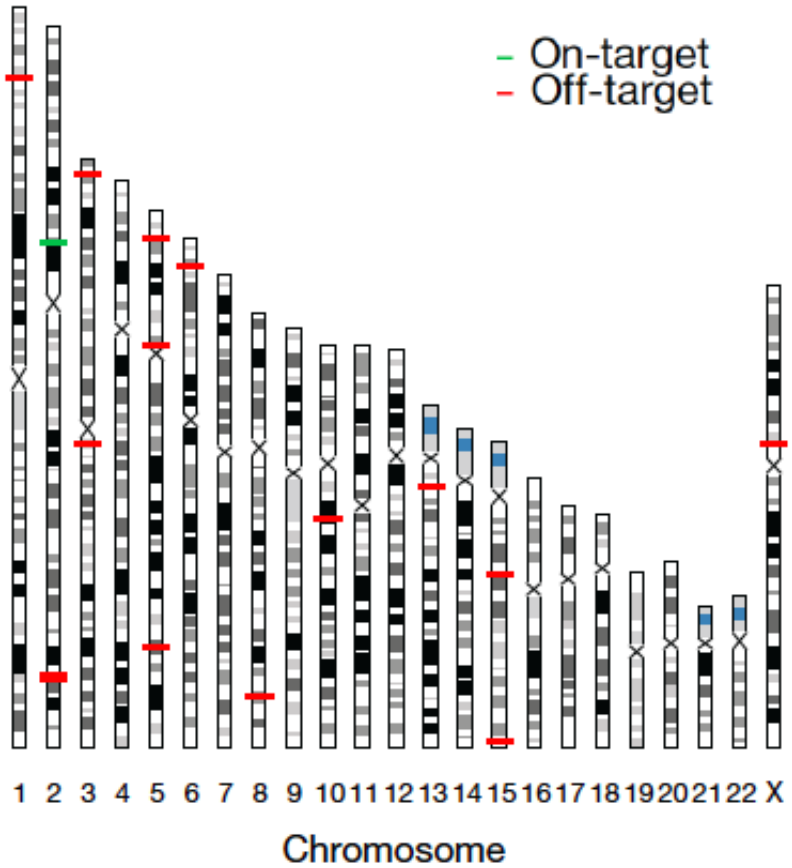


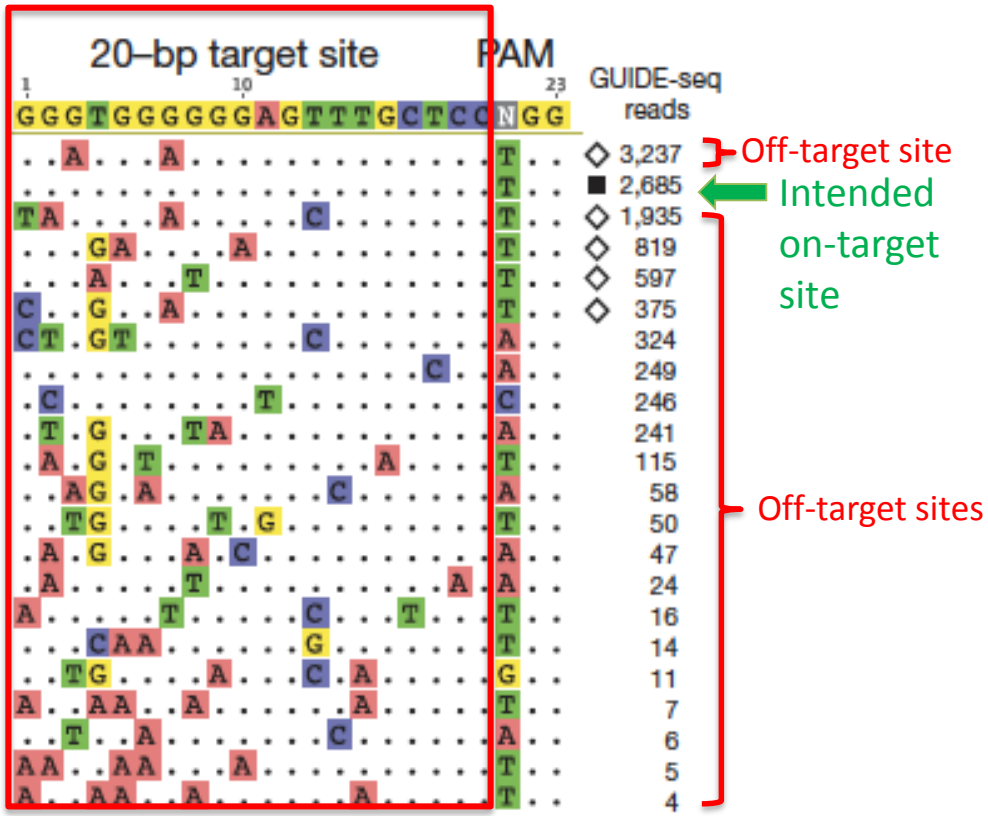
Off-target vs
On-target
Editing

ACS SynBio, 2017

Answer: In many cases it seems yes, but in some worst-case scenarios…

Genome-wide mapping

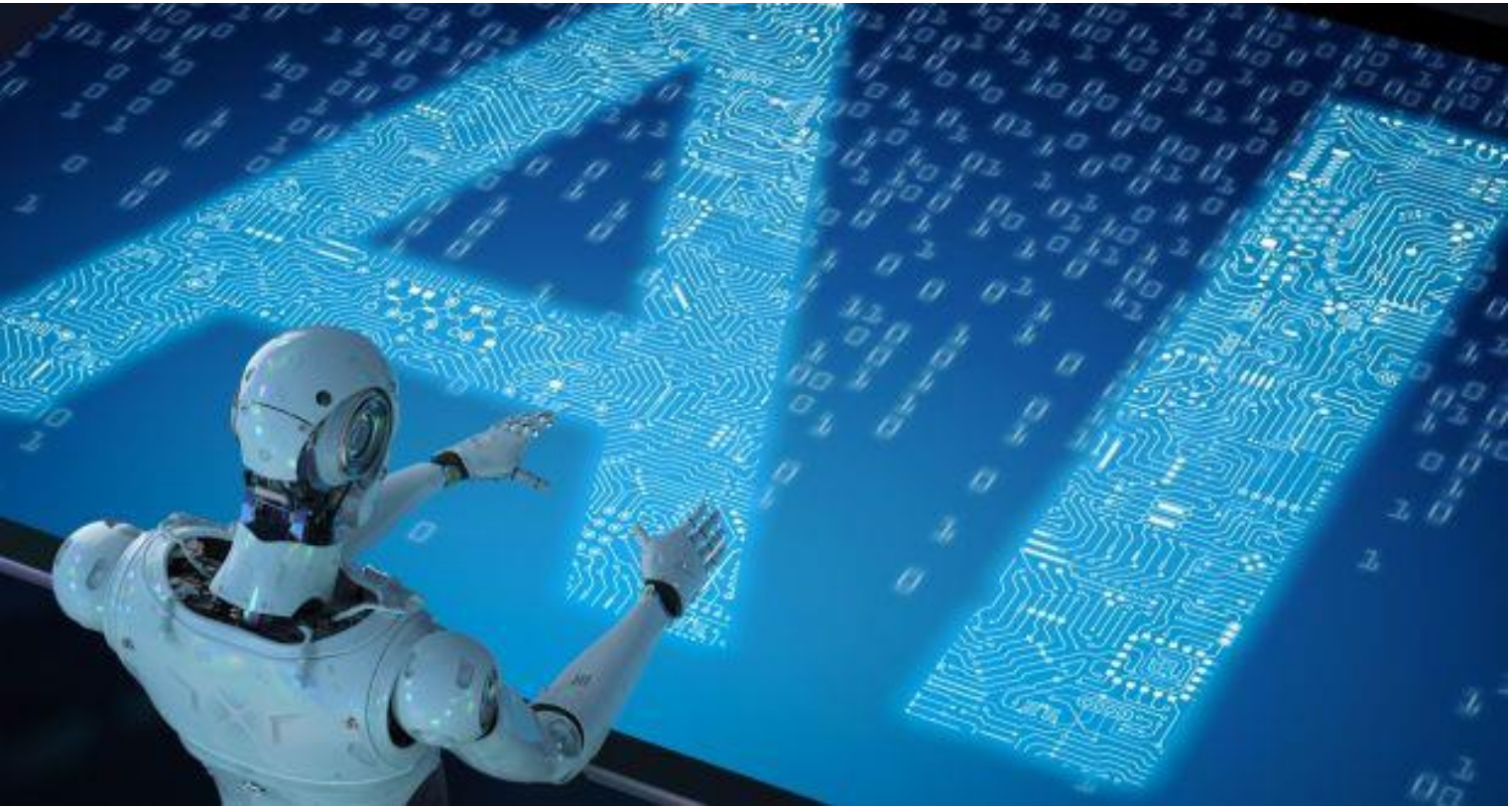




Nat. Biotech., 2014; Nature Rev. Genet. 2016;

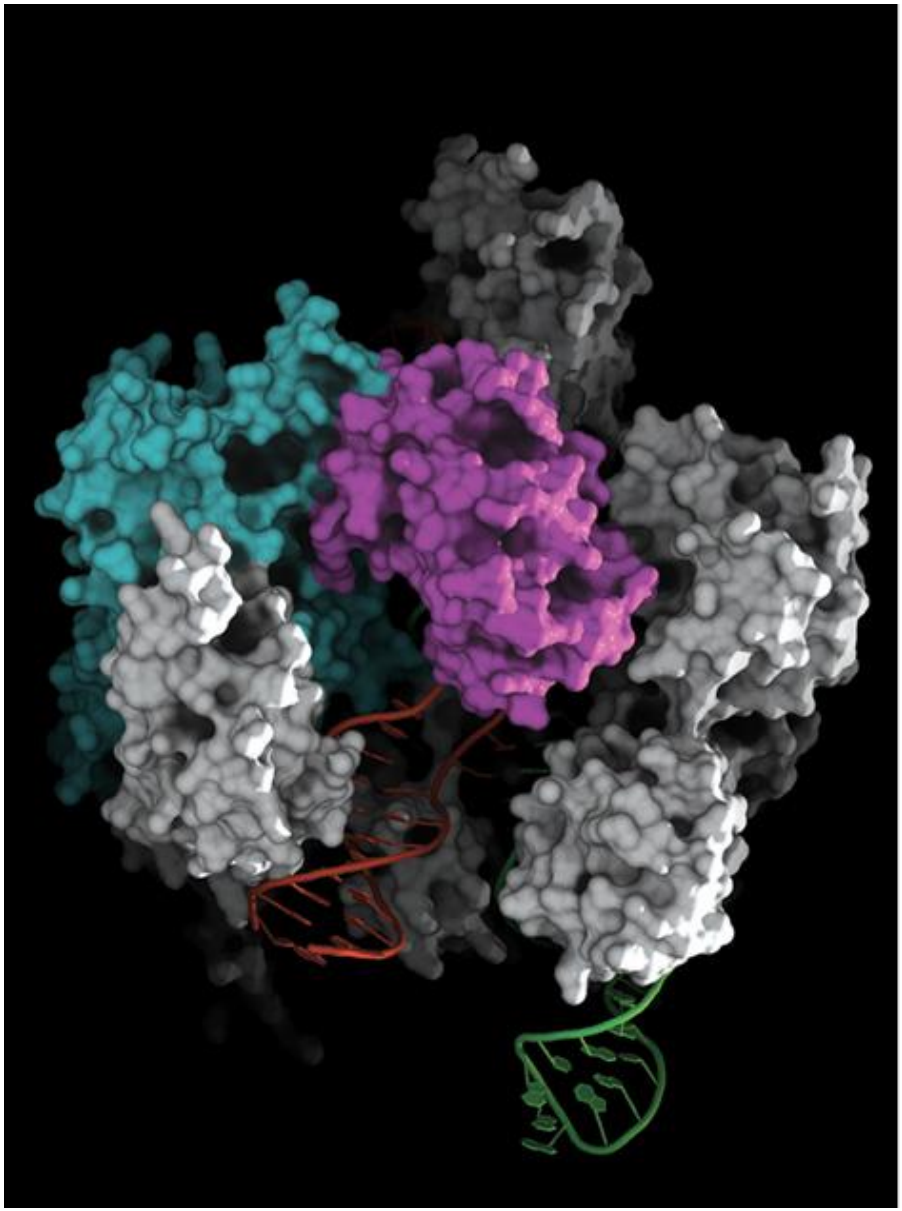
To improve targeting specificity (lower the off-target effects)

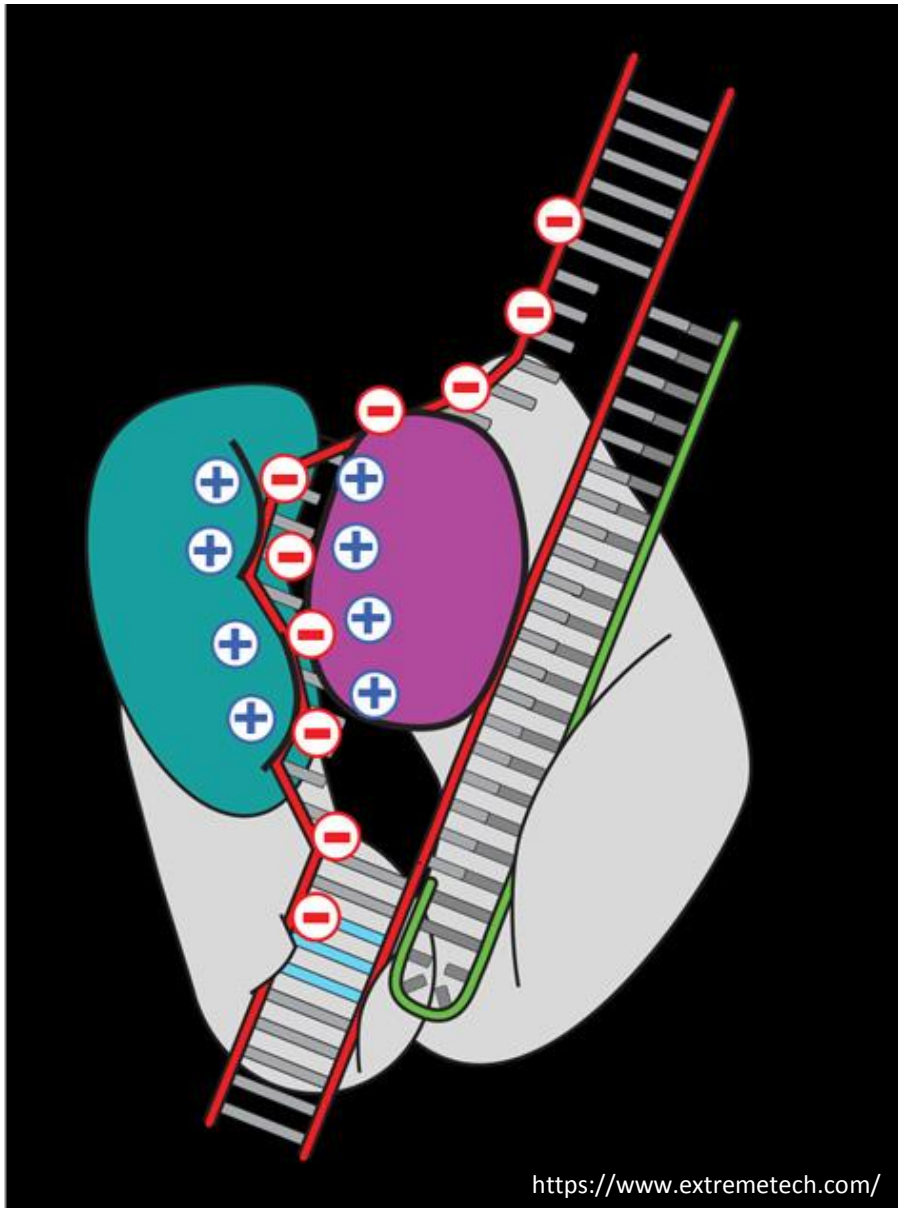
1. *In-silico* design of sgRNA with high on-target and low off-target activities



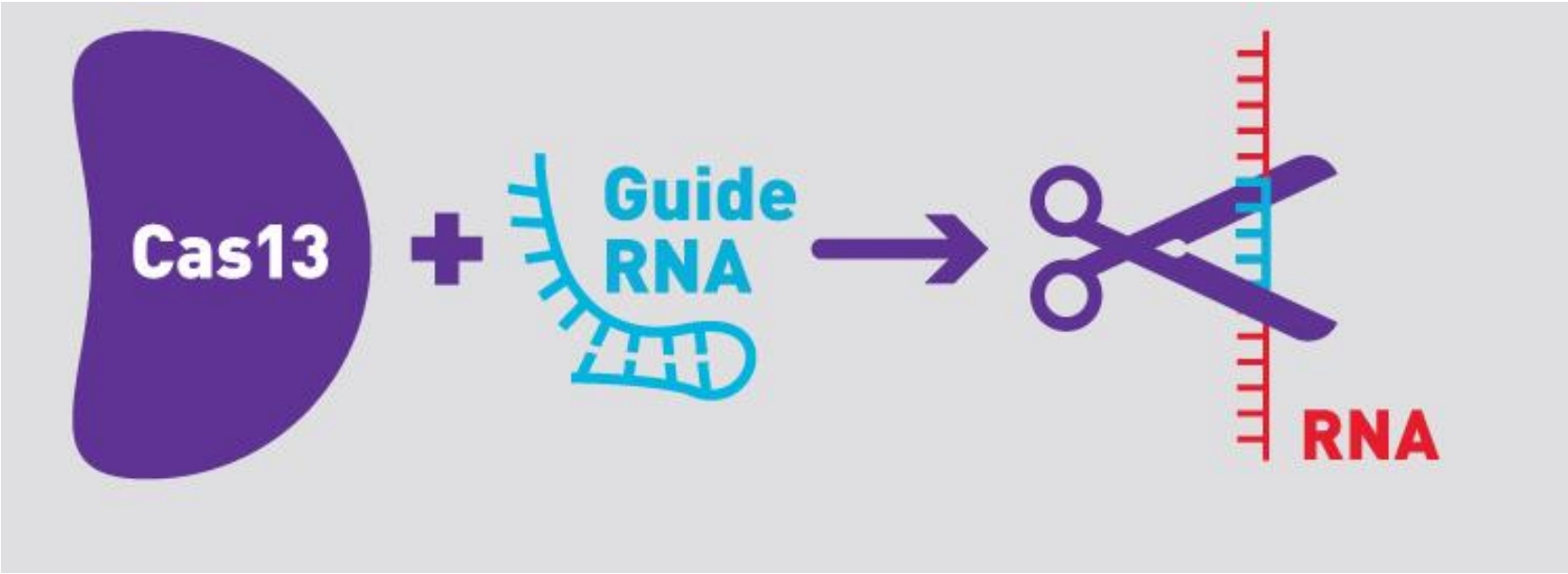
https://www.irishtimes.com/
https://propakistani.pk/2018

2. Modify Cas9 protein to reduce off-target activity



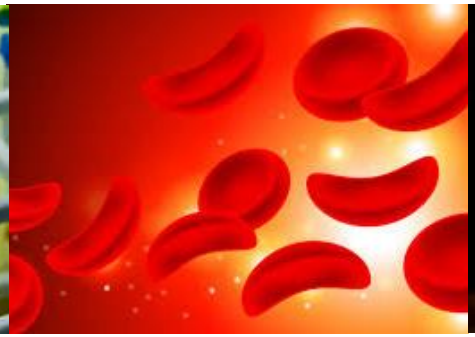


~~DNA~~ RNA editing

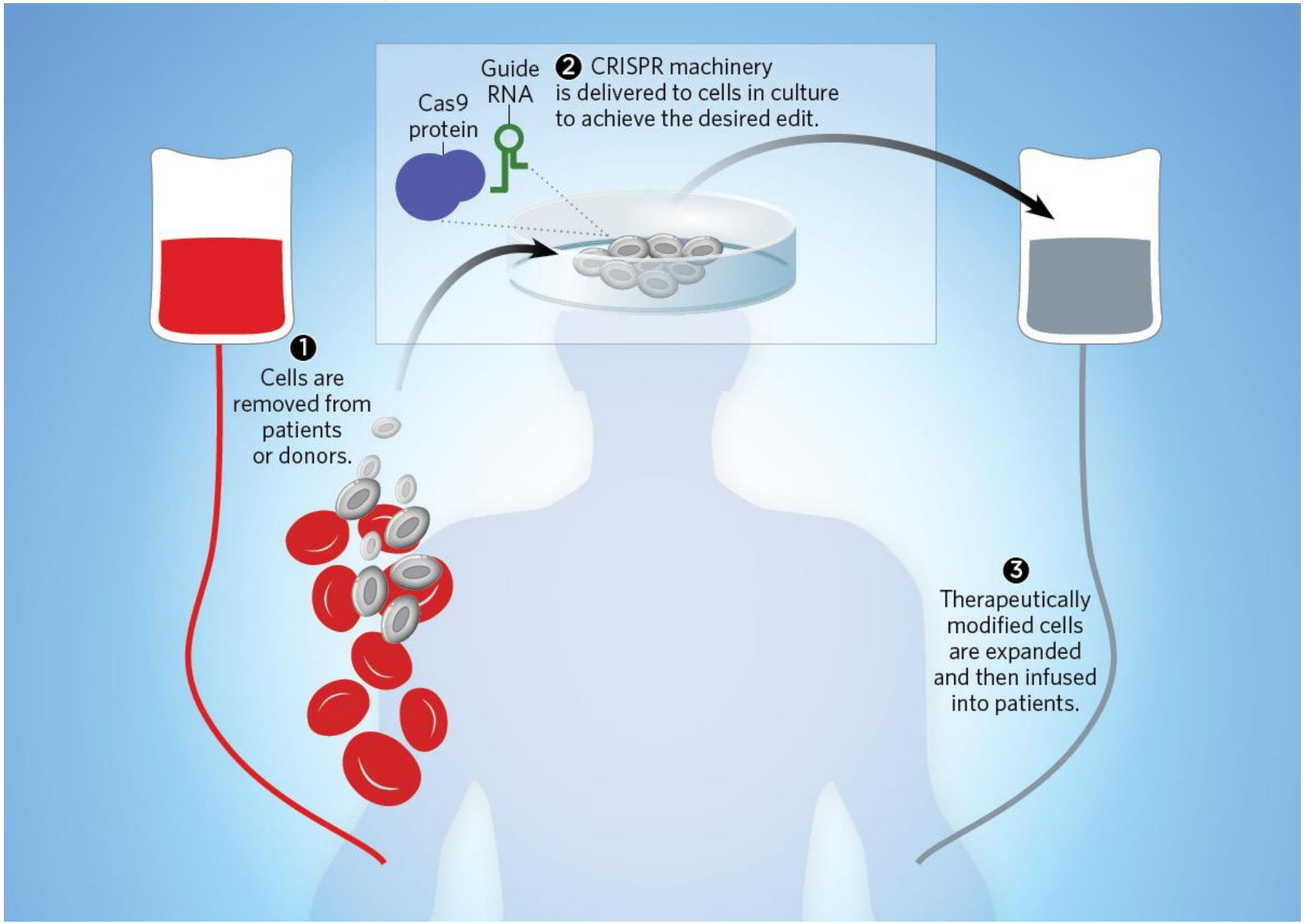


https://www.broadinstitute.org/

From tools to applications

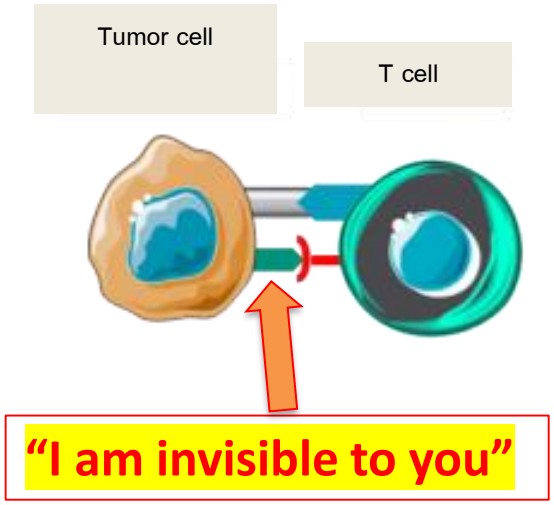


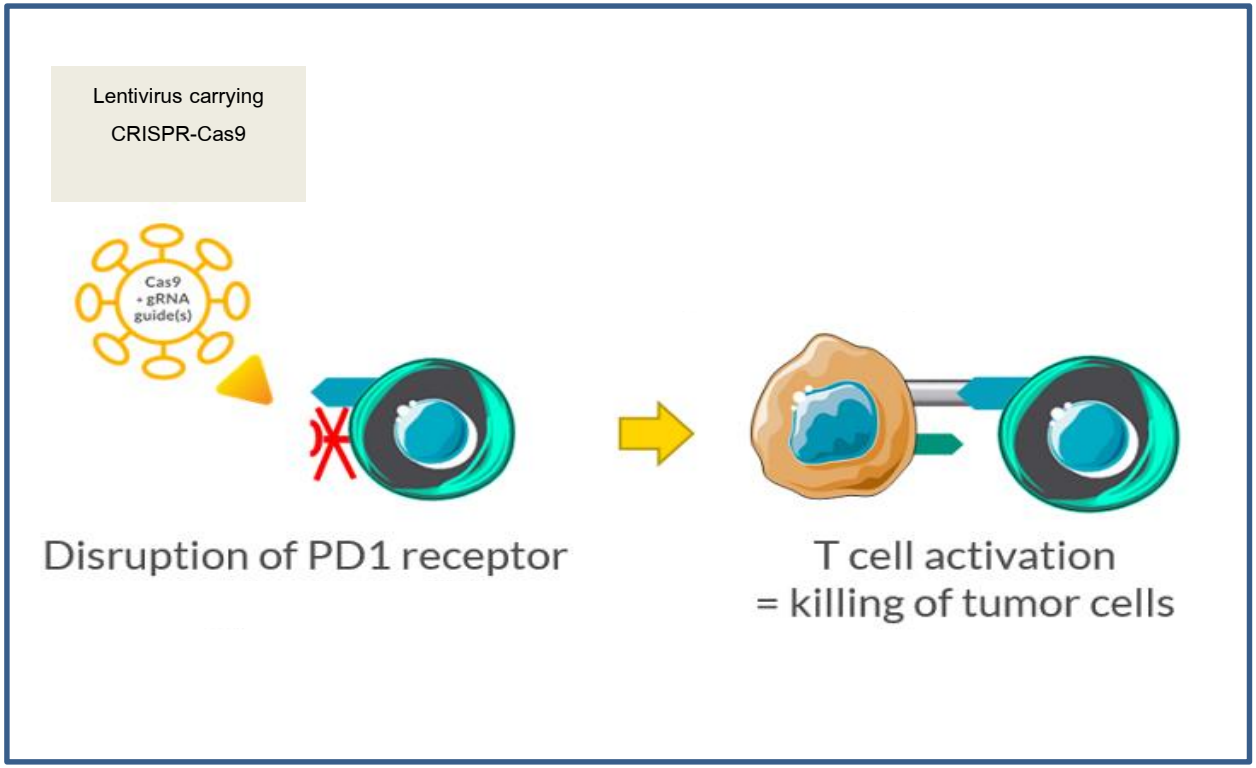
Ex vivo editing therapy



https://www.the-scientist.com/

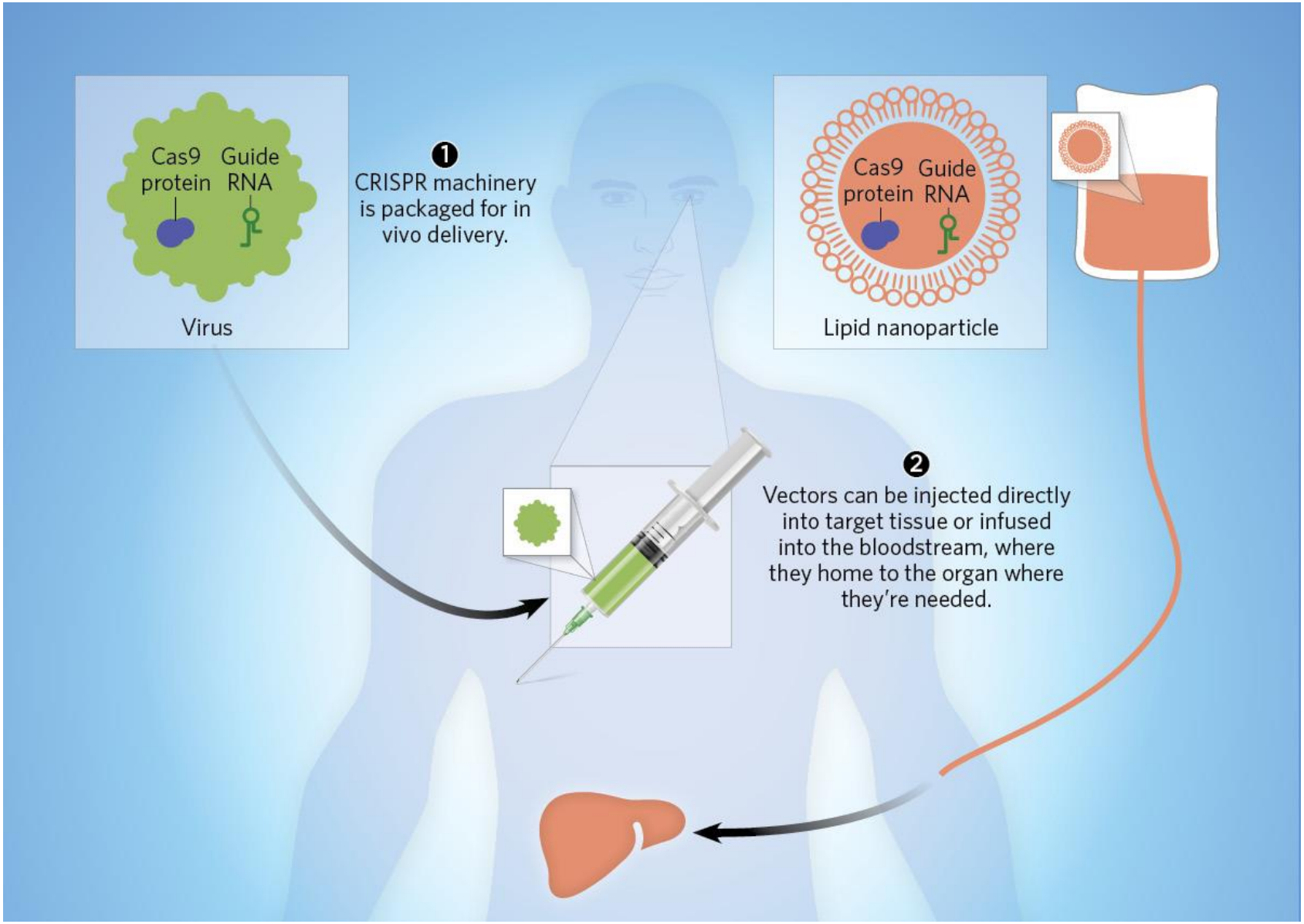
CRISPR for cancer immunotherapy





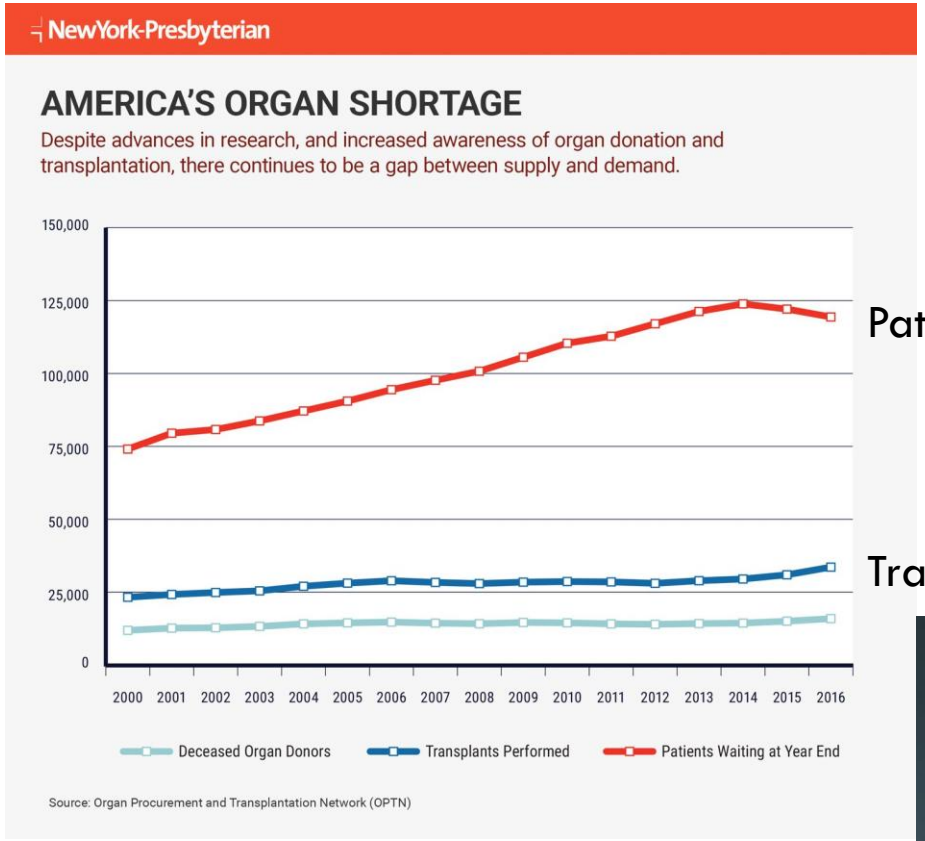
https://www.vectalys.com/applications-of-lentiviral-technologies/gene-editing/

In vivo editing therapy



https://www.the-scientist.com/

A long-standing challenge of organ transplantation



Patients waiting at year end

A big gap

Transplants performed

https://healthmatters.nyp.org/

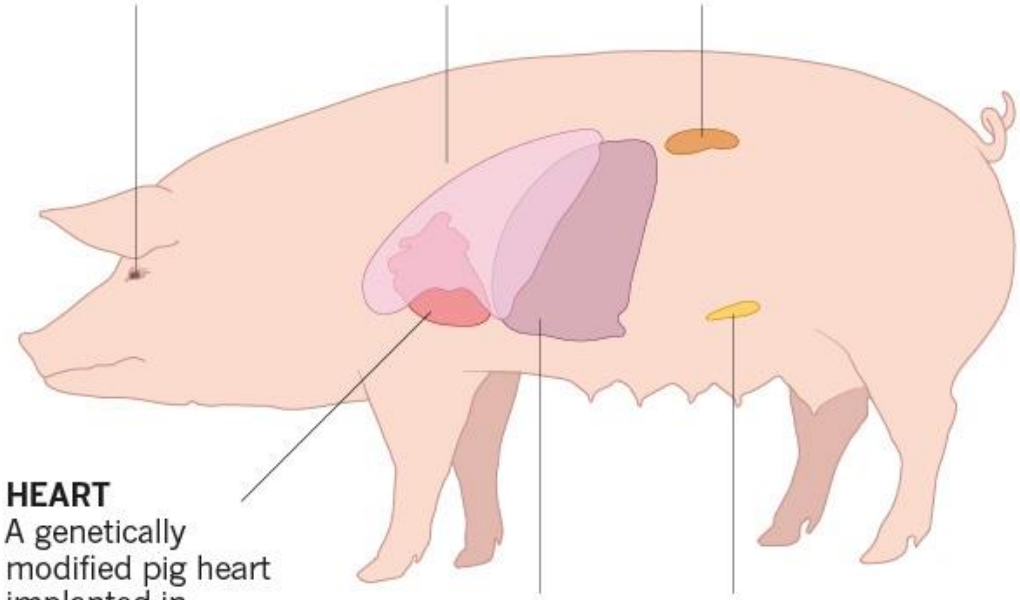
CHOICE CUTS

Researchers are looking to source an increasing variety of living tissues, including solid organs, from pigs. Many are attempting to genetically engineer the animals to reduce the risk of rejection and infection in humans.

CORNEA
Pig corneas were approved for marketing in China in April.

LUNG
A factory farm is being designed to produce 1,000 pig lungs per year.

KIDNEY
A kidney with six genetic modifications supported a baboon's life for 4 months.



HEART
A genetically modified pig heart implanted in a baboon's abdomen survived for 2.5 years.

LIVER
Livers could be engineered to produce their own antibodies against primate immune cells.

PANCREAS
Phase III clinical trials of insulin-producing islet cells are under way.

https://chilonas.com/2015

Oct 14, 2015

CRISPR-Clean Pig Genome Could Mean Safer Pig-to-Human Transplants

"Before pig organs can be made safe for transplantation into human patients, two problems need to be solved. Both problems, ultimately, come down to features of the pig genome. First, the pig genome contains genes that give rise to proteins that the human immune system will not tolerate. Second, it contains retroviruses that could be transmissible to humans. The retrovirus problem, at least, may soon be tractable, thanks to the CRISPR gene-editing technique."

The CRISPR technique has been used to inactivate all 62 copies of a retrovirus in pig

http://www.genengnews.com/

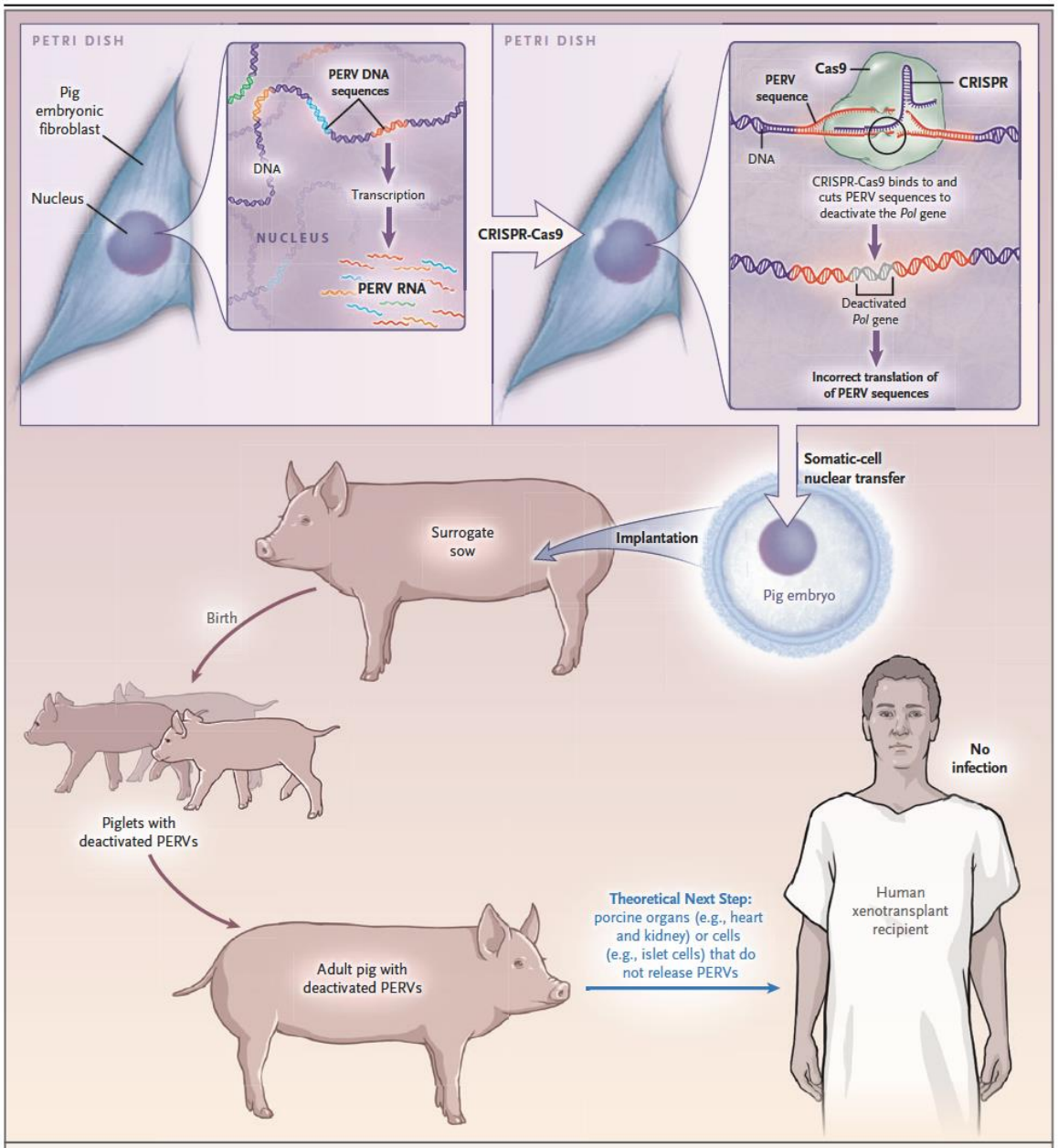


Figure 1. Toward Xenotransplantation — Genome Editing of the Pig.
Niu et al.[7] recently reported the generation of pigs in which all porcine endogenous retroviruses (PERVs) had been inactivated. Using a method called CRISPR (clustered regularly interspaced short palindromic repeats)–Cas, they inactivated the PERV polymerase gene, thus preventing the release of infectious viruses. They then transferred the nuclei of these treated cells into pig oocytes, giving rise to embryos that were then transferred to surrogate sows. This process resulted in the birth of healthy piglets with inactivated PERVs.

Without CRISPR, deleting multiple genes or swapping them for their human counterparts takes many more generations and time.

New England Journal of Medicine, 2017

A feasible/sustainable plan to end malaria with CRISPR-edited mosquitoes?

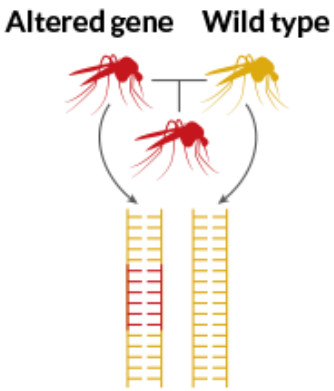
"Decades of malaria control has taught us that the most effective strategy to prevent malaria is to control the mosquito itself."

Only female mosquitoes bite humans, and it will pass the infection onto the bitten person. A gene drive was designed in laboratory to target genes that upon disruption make the female mosquitoes unable to bite.

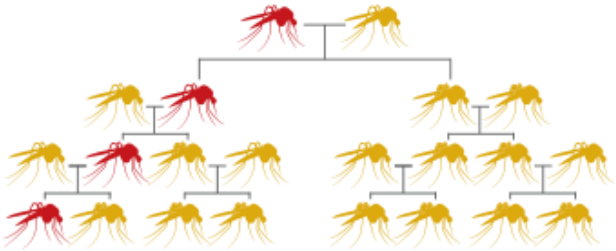
http://theconversation.com/

What is gene drive?

Normal inheritance

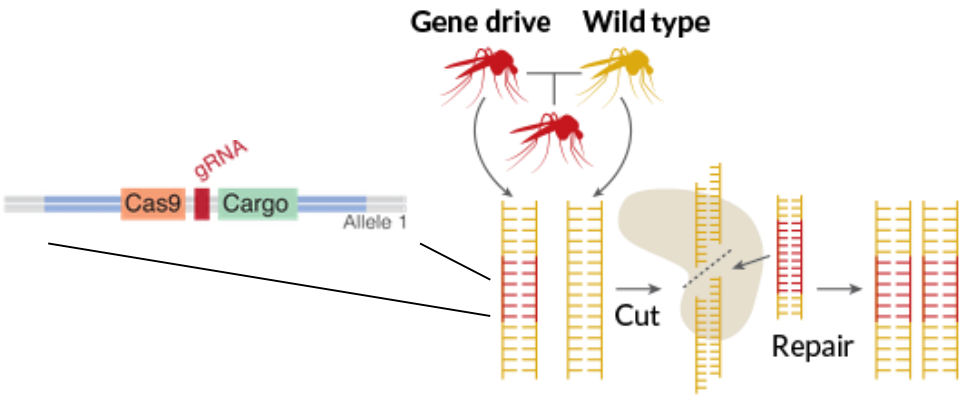


Altered gene without gene drive: One copy inherited from one parent. 50 percent chance of passing it on.

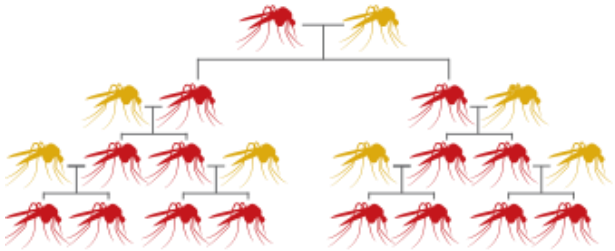


Altered gene does not spread

Gene drive inheritance



Altered gene as gene drive: One copy converts gene inherited from other parent. More than 50 percent chance of passing it on.



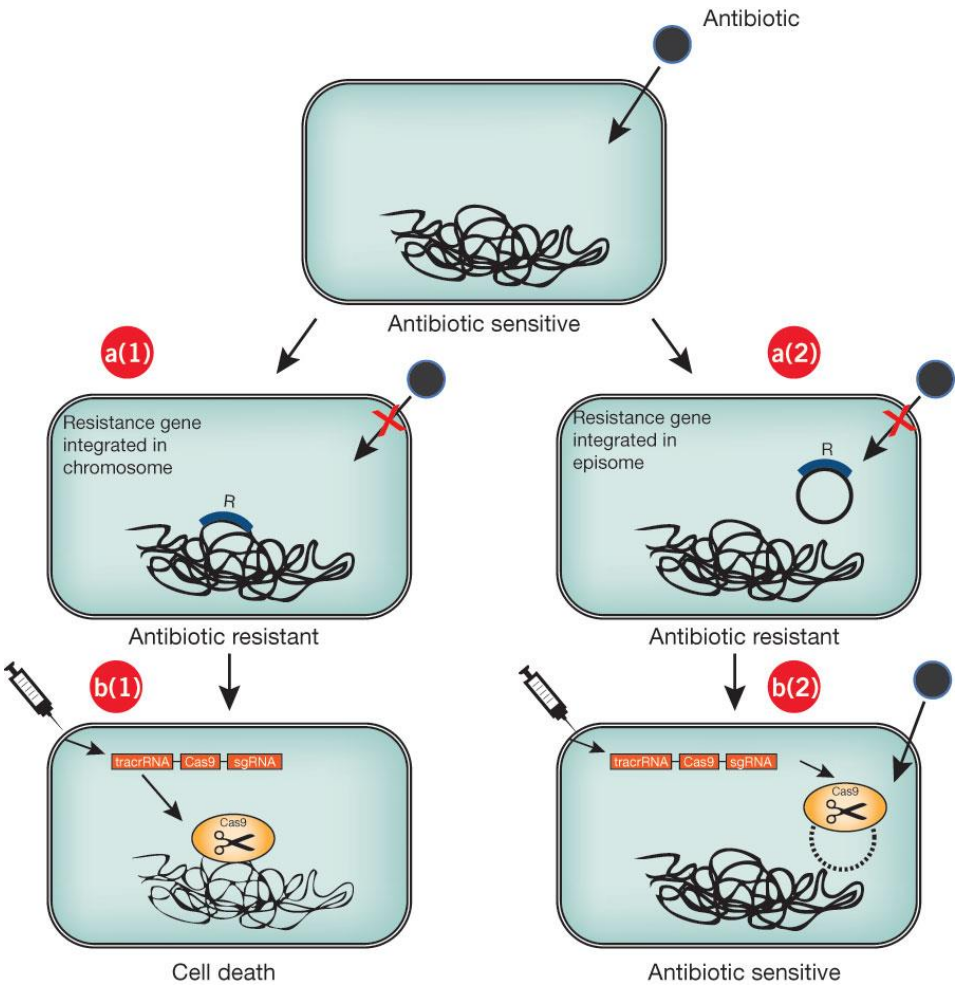
Altered gene is almost always inherited

http://theconversation.com/; https://www.sciencenews.org/

New quick ways to tackle antibiotic resistance?

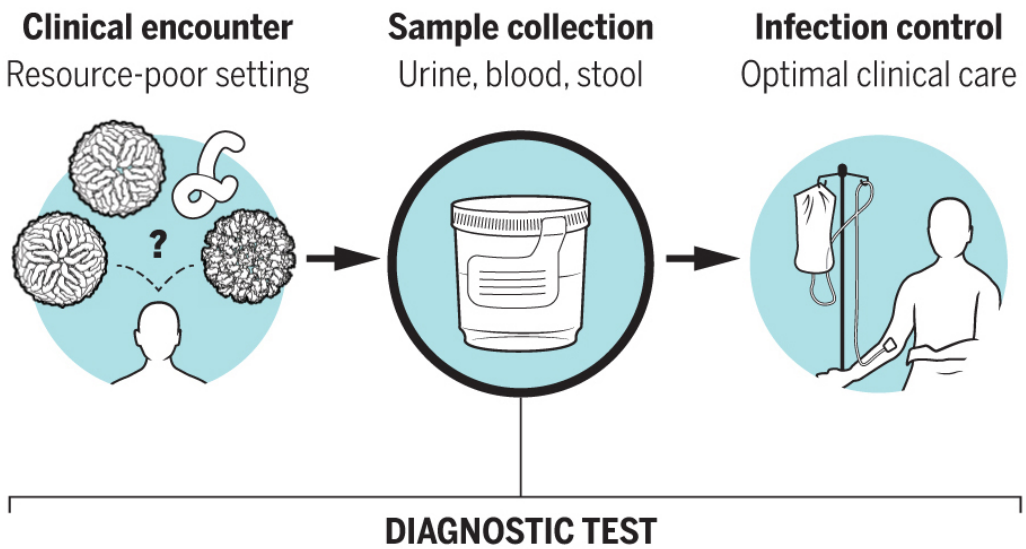
Challenge:
No new classes of antibiotic have been developed for more than 25 years. We have not kept pace with the ability of many pathogens to develop antibiotic resistance.

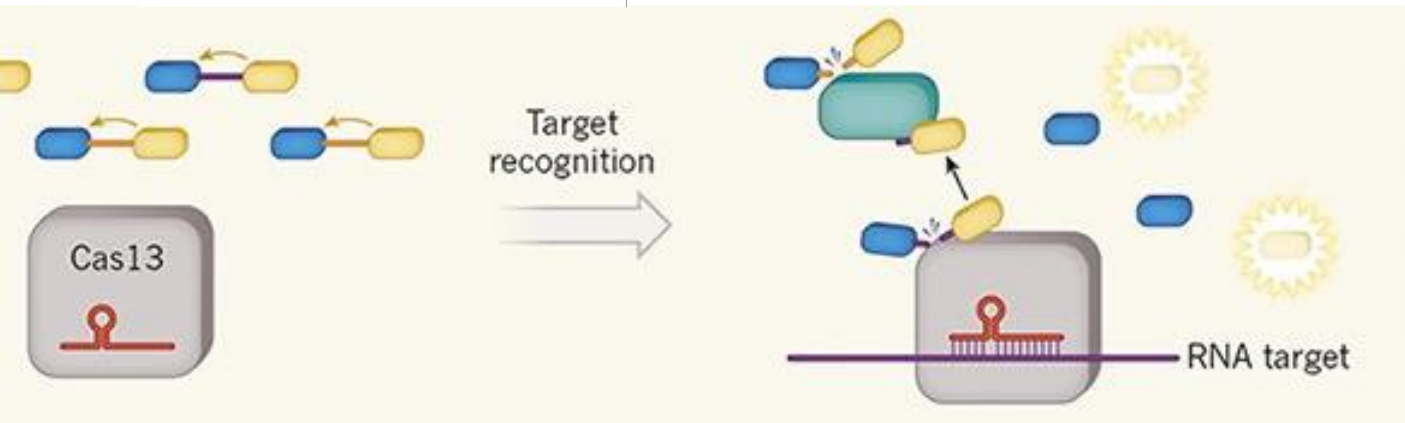
Possible solution:
Design and deliver CRISPR systems that target bacteria that contain antibiotic resistance genes or virulence genes.

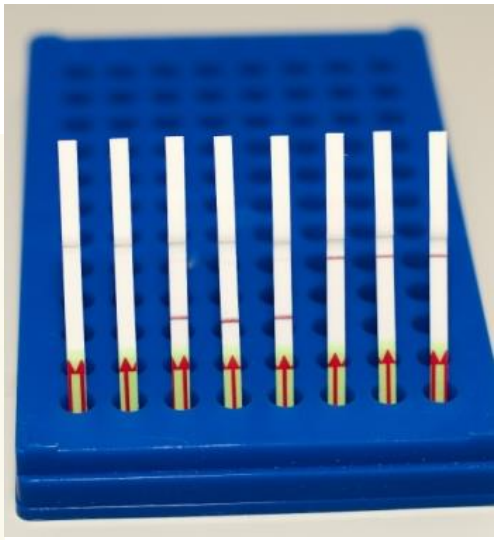


http://www.nature.com/scibx/

CRISPR diagnostics

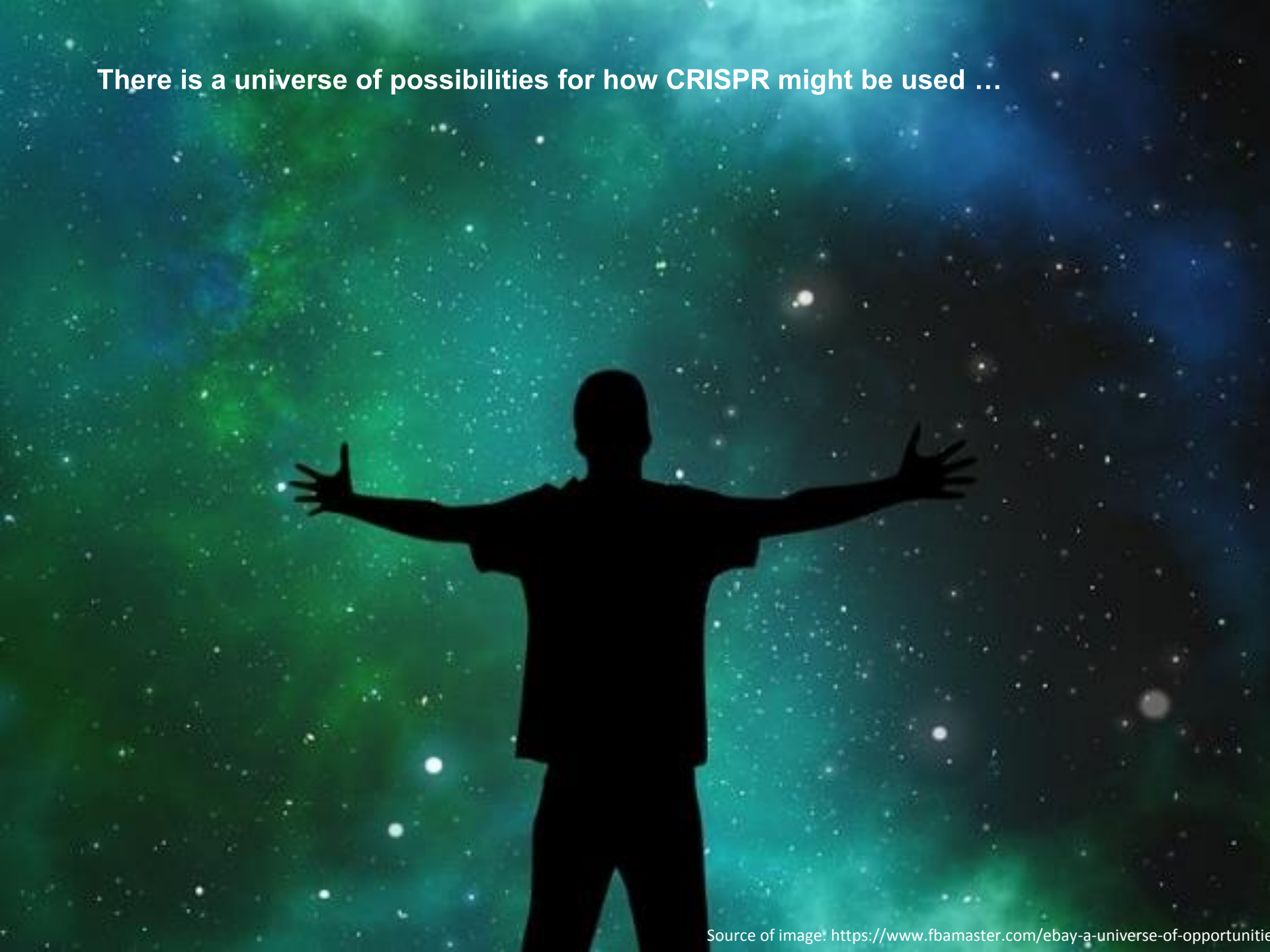






Science, 2017; 2018; https://twitter.com/NatureNV/status/993195189404549121; https://www.burnet.edu.au/

There is a universe of possibilities for how CRISPR might be used …



Source of image: https://www.fbamaster.com/ebay-a-universe-of-opportunitie

Planning your CRISPR experiment?

What is the **biological question** that you want to address?

Select your desired manipulation. **Knock out? Edit? Activation? Repression?**

Genome-wide, single, or multiple edit?

Select the expression and delivery system. **Design gRNA.** Deliver CRISPR components into cells.

https://blog.addgene.org/crispr-101

https://130.med.hku.hk/


WITH
GREAT POWER COMES
GREAT RESPONSIBILITY