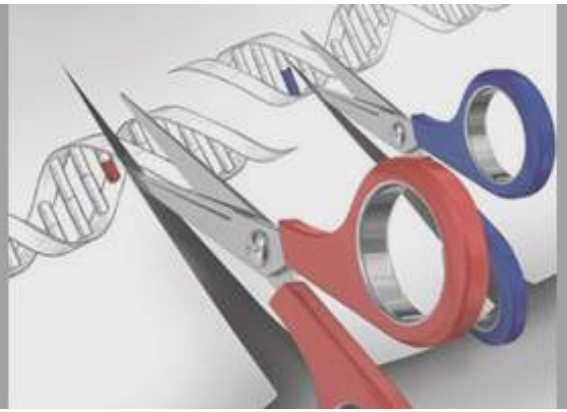
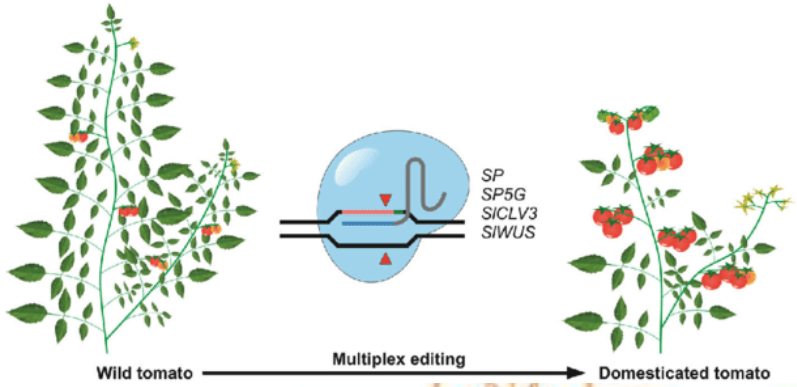
Genetika EA (BSc)
Genomszerkesztés

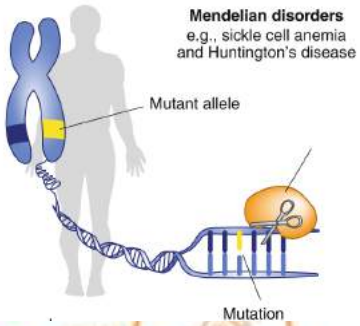


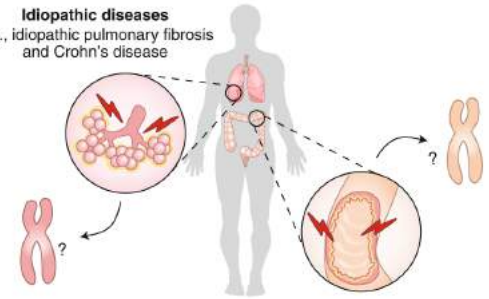
Varga Máté (Genetikai Tanszék)
mvarga@ttk.elte.hu

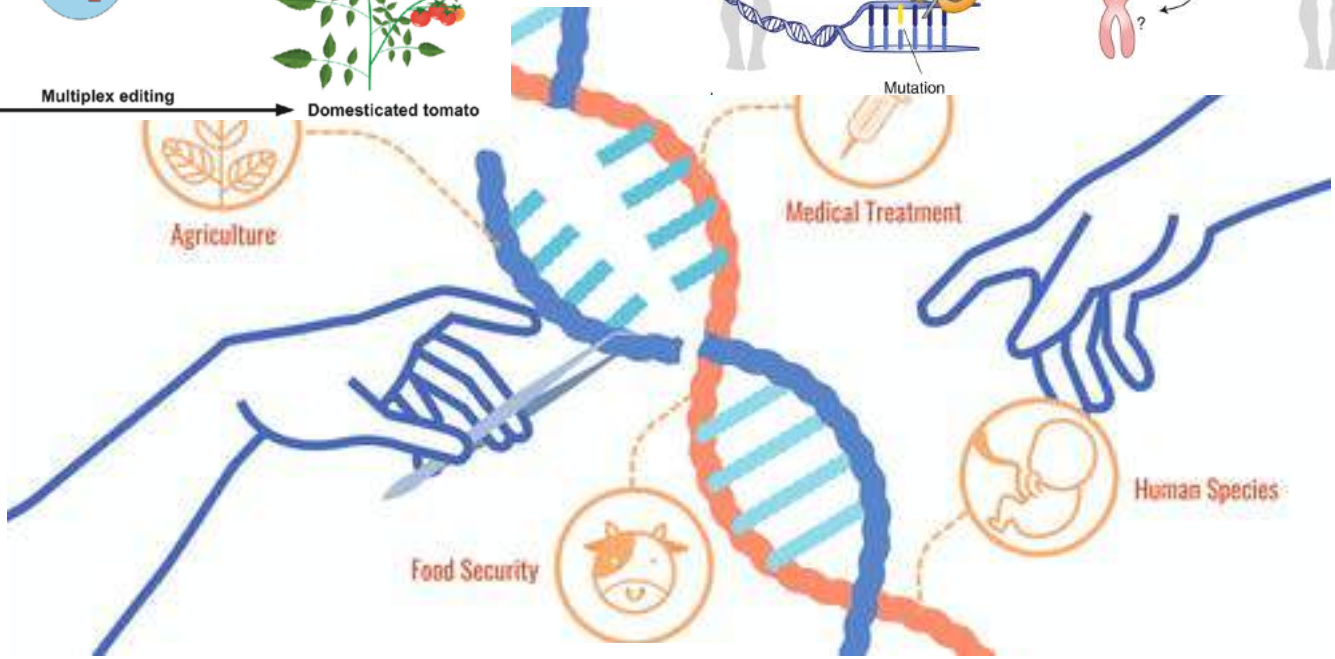
2020.10.21.

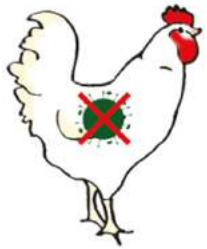
Miért akarunk „genomszerkeszteni”?



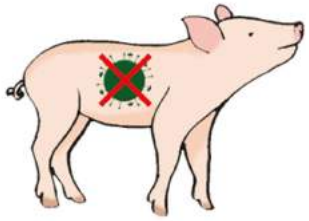








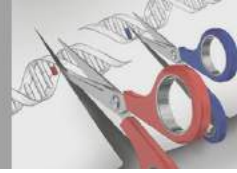
Resistant chicken

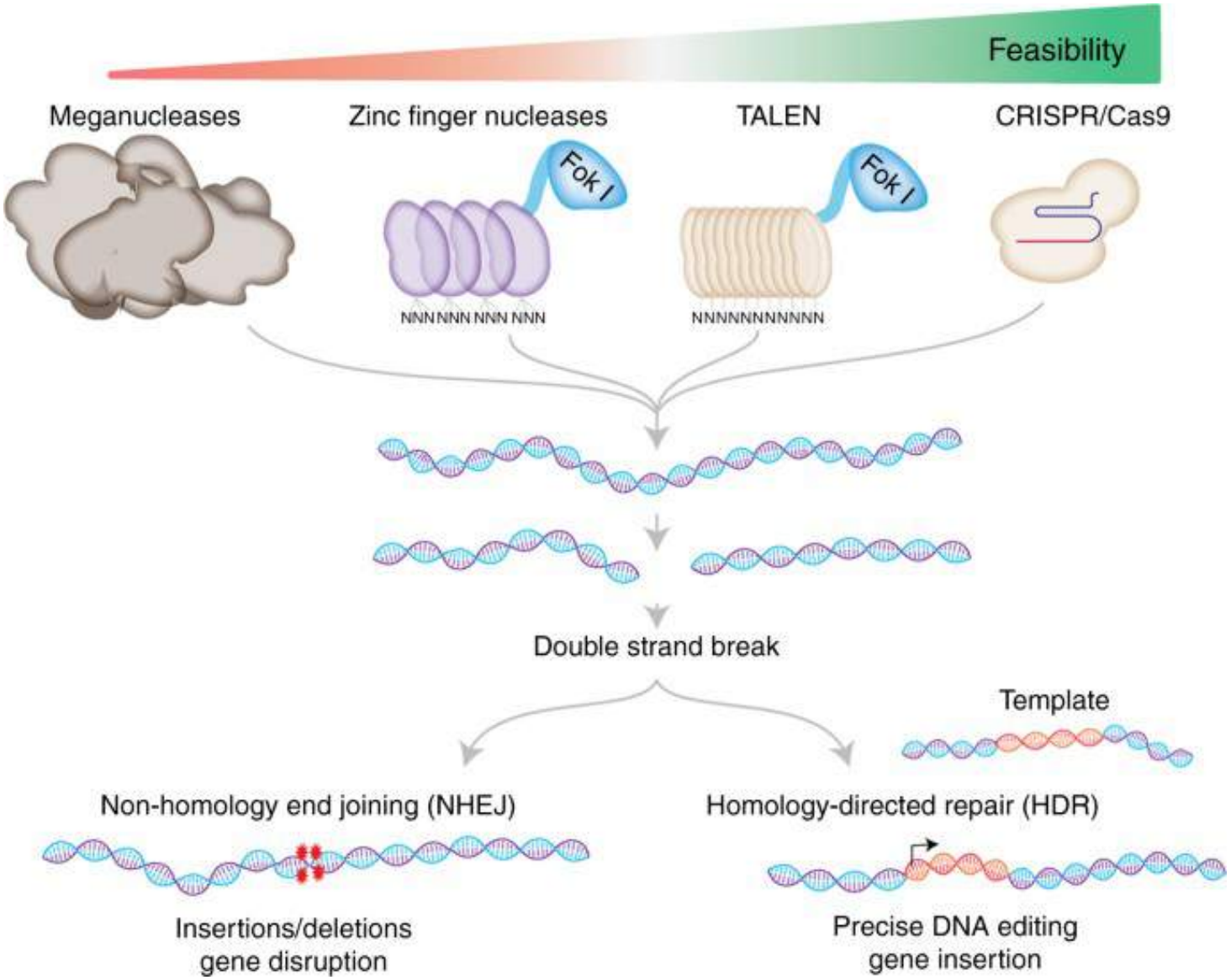


Resistant pig

(The Conversation)

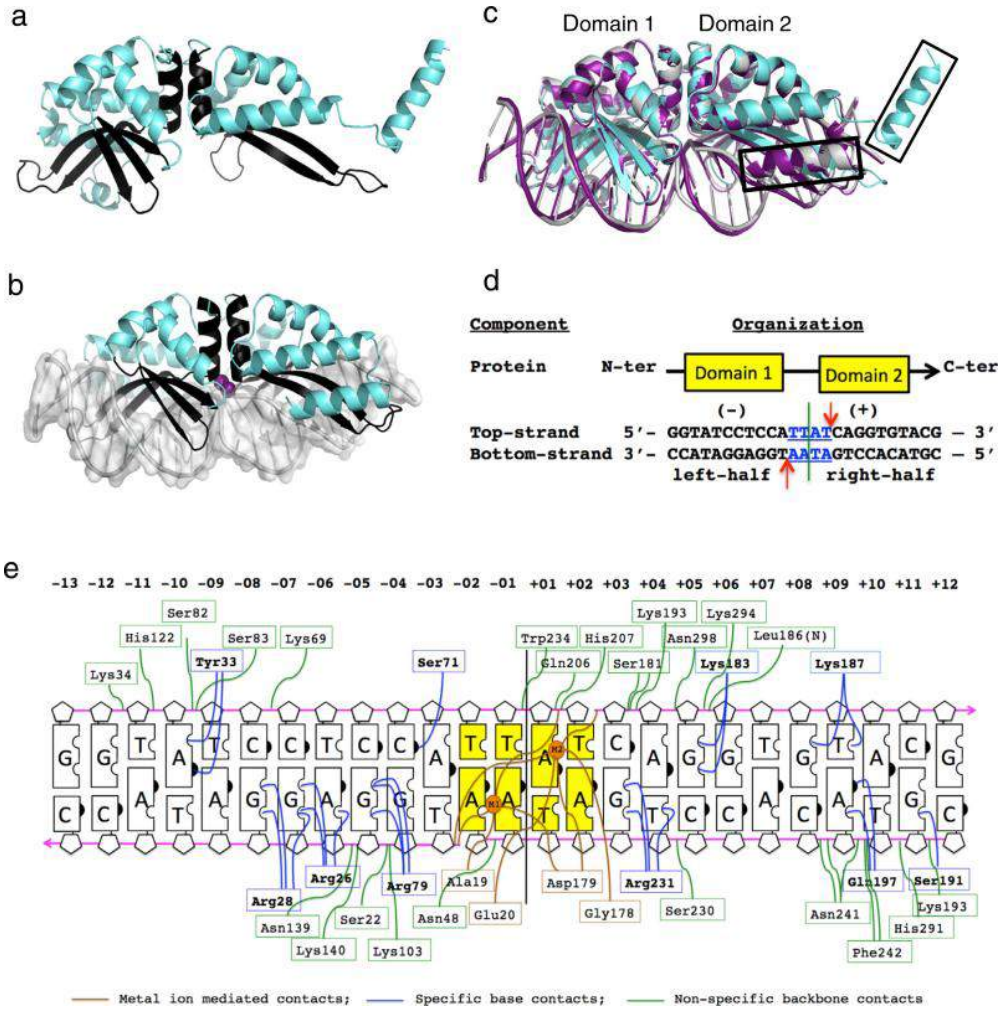
Genomszerkesztés a sejt szemszögéből: DNS hibajavító mechanizmusok





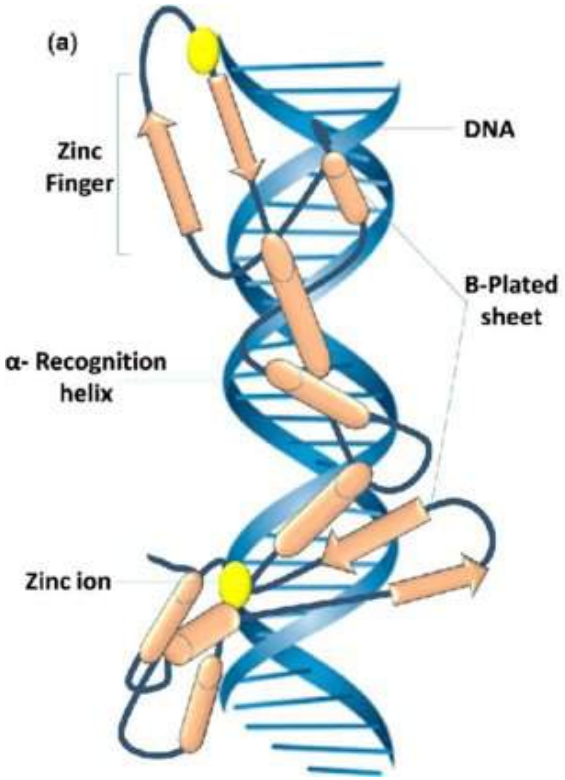
(Wikipedia)

DNS-specifikus kötés: meganukleázok

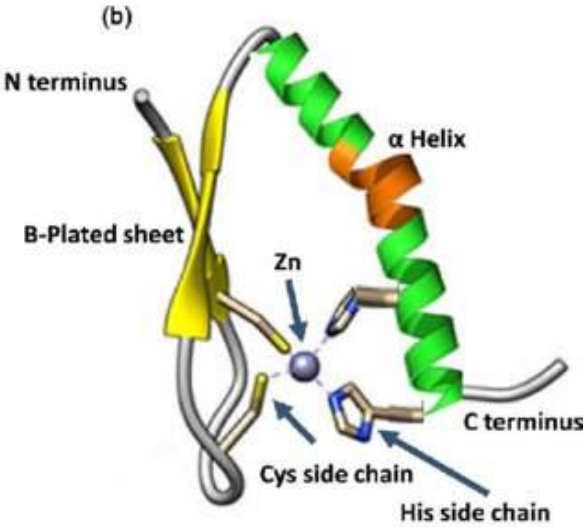


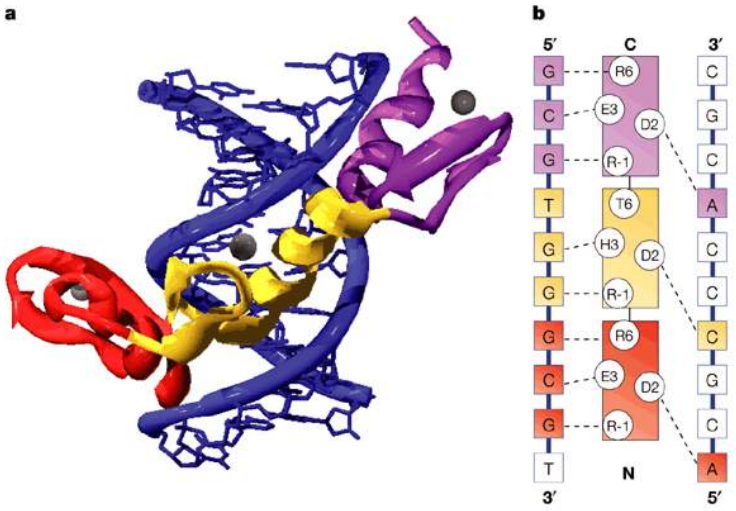
(Shen et al. 2015 *J Mol Bio*)

DNS-specifikus kötés: ZFN-nukleázok



(Aslam et al. 2019 *Am J Mol Bio*)

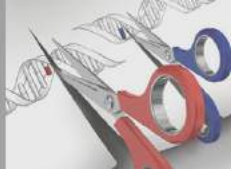


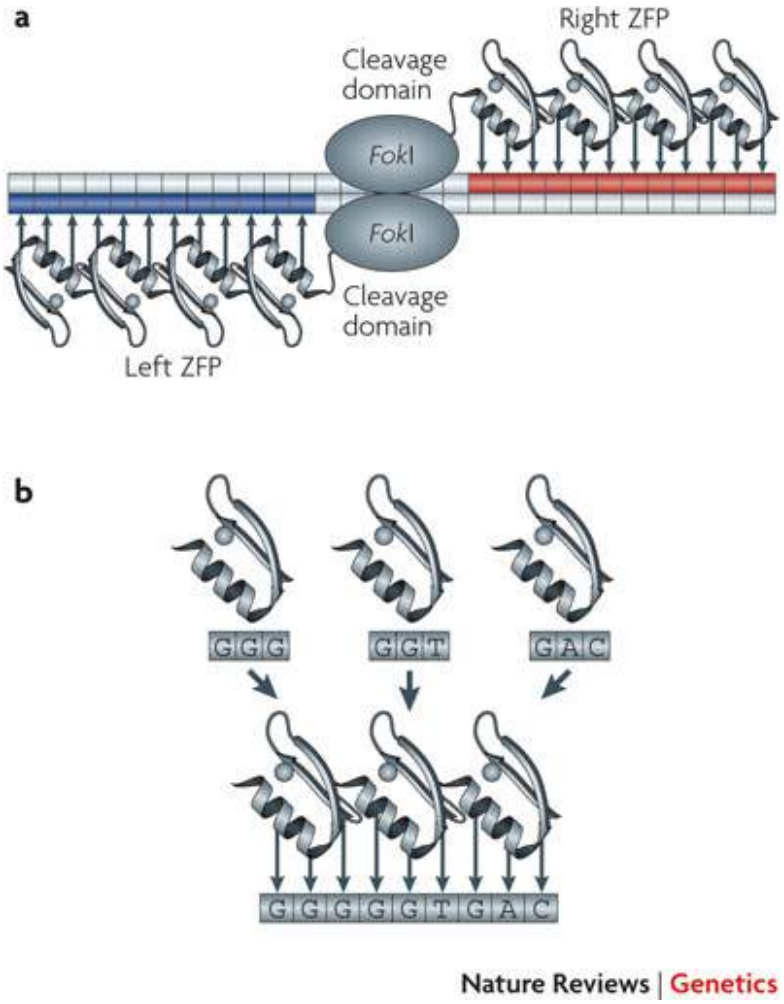


(Jamieson et al. 2003)

Nature Reviews | Drug Discovery

DNS-specifikus kötés: ZFN-nukleázok

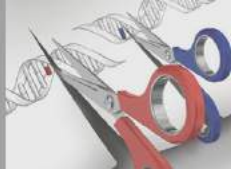




Nature Reviews | Genetics

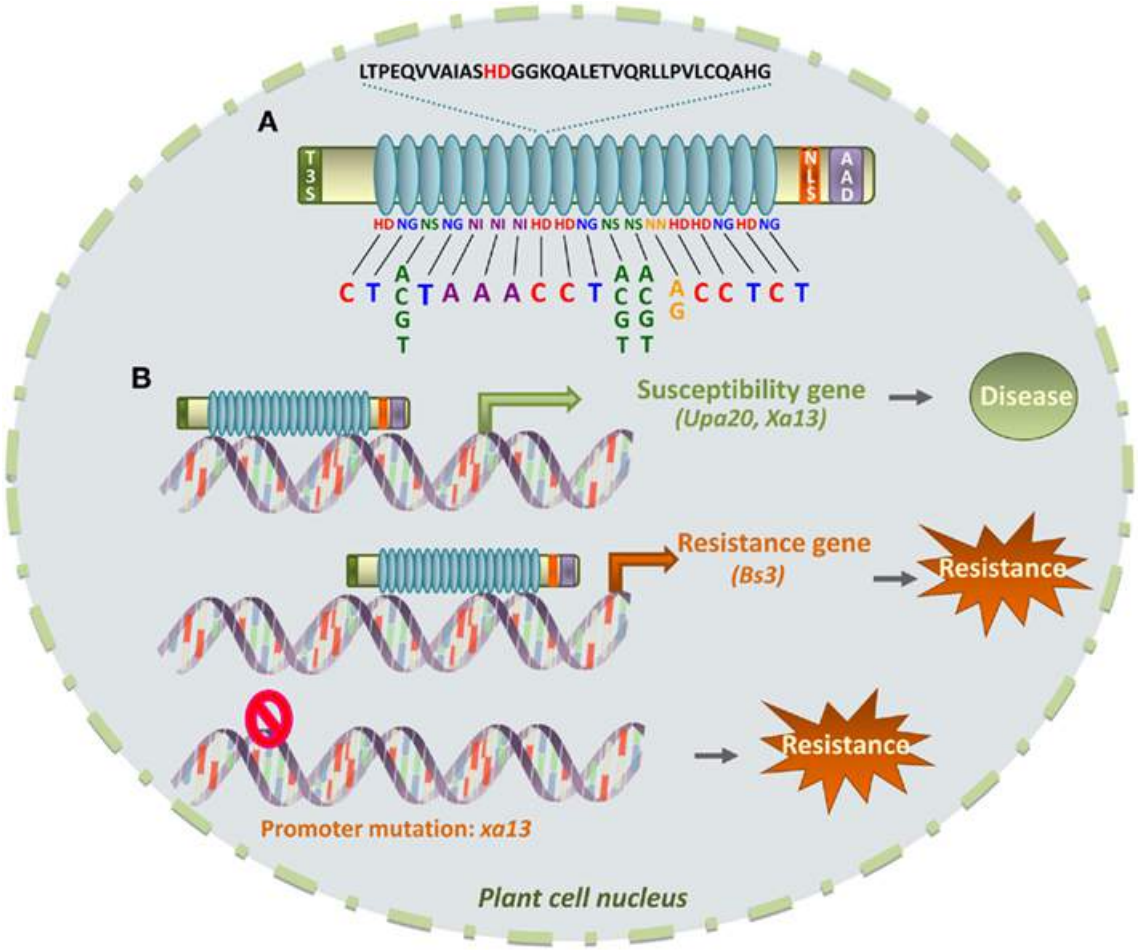
(Urnov et al. 2010)

DNS-specifikus kötés: TALE nukleázok



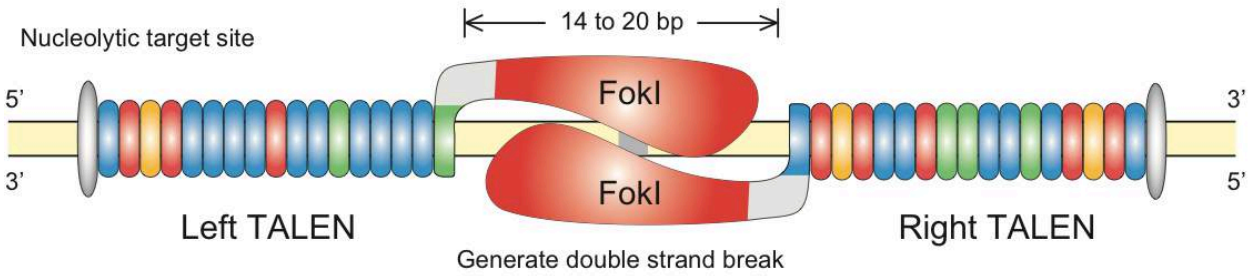
- *Xanthomonas* nevű növényi patogénből izolálták

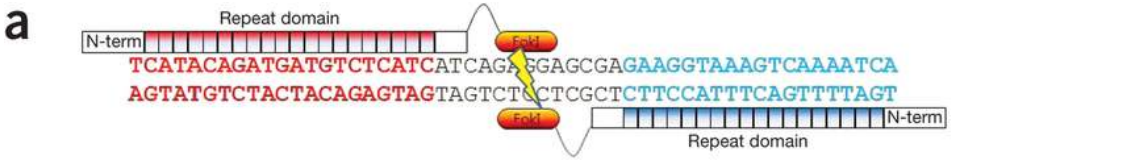
TALE = Transcription Activator Like Effectors

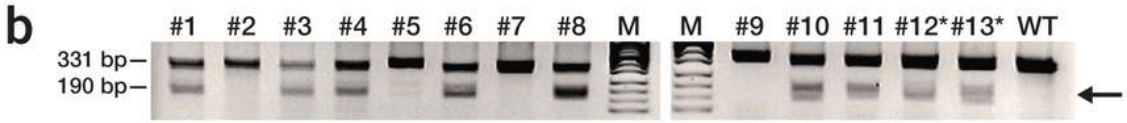


(Rivas and Genin, 2011, *Front Plant Sci*)

DNS-specifikus kötés: TALE nukleázok





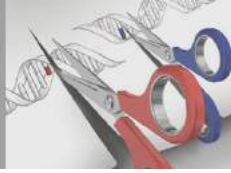


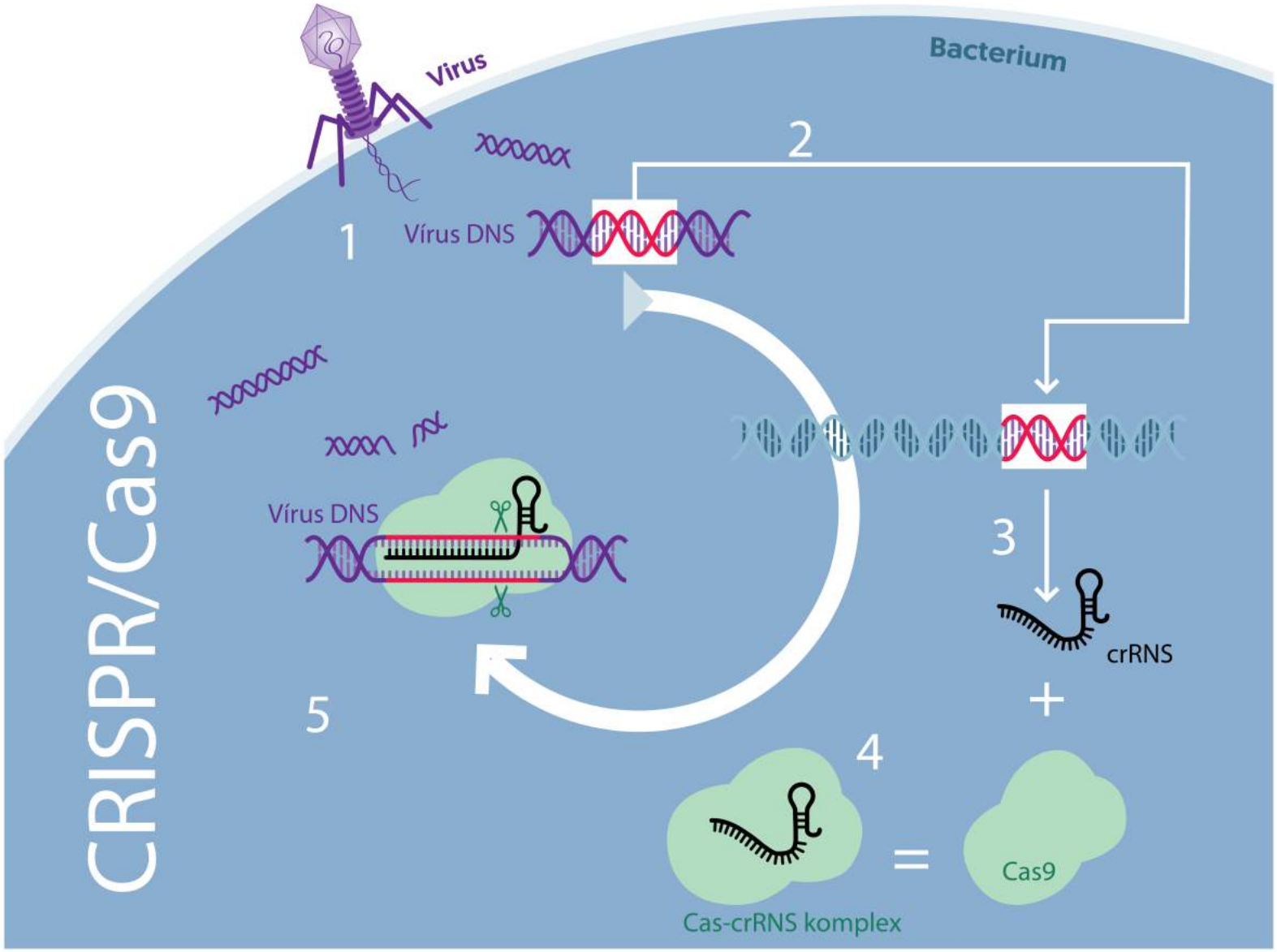
c

		H T D D V S S S E E R E G K V K I	Amino acid sequence
		T CAT ACA GAT GAT GTC TCA TCA TCA GAG GAG CGA GAA GGT AAA GTC AAA ATC A	Wild-type sequence
Founders	#1	T CAT ACA GAT GAT ac- --- --- --- --- --- --- --A GGT AAA GTC AAA ATC A	Δ21 bp + aa change
		T CAT ACA GgT GAT G-- --- --- --- --- --- --- -AA GGT AAA GTC AAA ATC A	Δ21 bp + aa change
	#3	T CAT ACA GAT GAT GTC TCA TCA TCA GAG --- CGA GAA GGT AAA GTC AAA ATC A	Δ3 bp
		T CAT ACA GAT GAT GTC TCA TCA TCA G-- --- CGA GAA GGT AAA GTC AAA ATC A	Δ5 bp (frameshift)
	#4	T CAT ACA GAT GAT GTC TCA TCA TCA G-G GAG CGA GAA GGT AAA GTC AAA ATC A	Δ1 bp (frameshift)
		T CAT ACA GAT GAT GTC TCA TCA TCA GAG GAG CGA GAA GGT AAA GTC AAA ATC A	WT
	#5	T CAT ACA GAT GAT GTC TCA TCA TC- --- -AG CGA GAA GGT AAA GTC AAA ATC A	Δ5 bp (frameshift)
		T CAT ACA GAT GAT GTC TCA TCA TCA GAG GAG CGA GAA GGT AAA GTC AAA ATC A	WT
	#6	T CAT ACA GAT GAT GTC TC- --- --- --- --G CGA GAA GGT AAA GTC AAA ATC A	Δ12 bp
		T CAT ACA GAT GAT G-- --- --- --- --- --- --- -AA GGT AAA GTC AAA ATC A	Δ21 bp
	#8	T CAT ACA GAT GA- --- --- --- --- --- --- --- --A GGT AAA GTC AAA ATC A	Δ24 bp
		T CAT ACA GAT GAT GTC Tac aga t-- --- --- --- GAA GGT AAA GTC AAA ATC A	Δ11 bp (frameshift)
	#10	T CAT ACA GAT GAT GTC TCA TCA TCA G-G GAG CGA GAA GGT AAA GTC AAA ATC A	Δ1 bp (frameshift)
		T CAT ACA GAT GAa --- --- --- --- --- --- --- --- --- --- AAA GTC AAA ATC A	Δ27 bp + aa change
	#11	T CAT ACA GAT GAT GTC TCA TCA TCA G-- GAG CGA GAA GGT AAA GTC AAA ATC A	Δ2 bp (frameshift)
		T CAT ACA GAT GA- --- --- --- --- --- --- --- --A GGT AAA GTC AAA ATC A	Δ24 bp

(Sung et al., 2013, *Nat Biotech*)

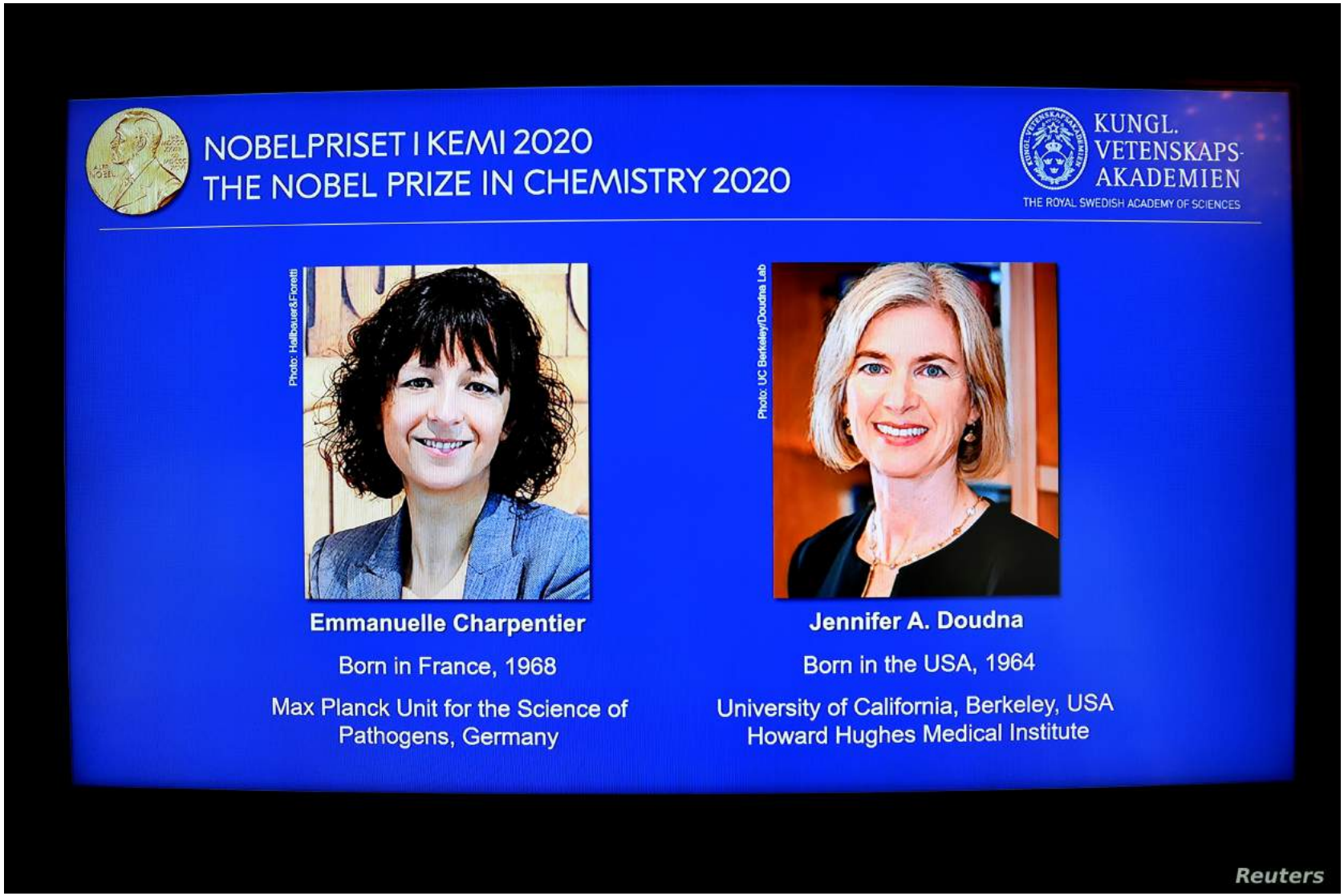
DNS-specifikus kötés: Cas nukleázok



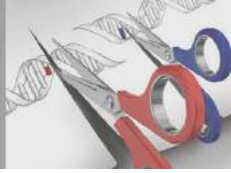


CRISPR = clustered regularly interspaced palindromic repeats

CRISPR alapú gén-editálás



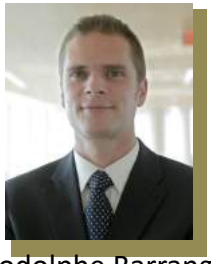
CRISPR alapú gén-editálás – a felfedezők




Yoshizumi Ishino


Francisco Mojica

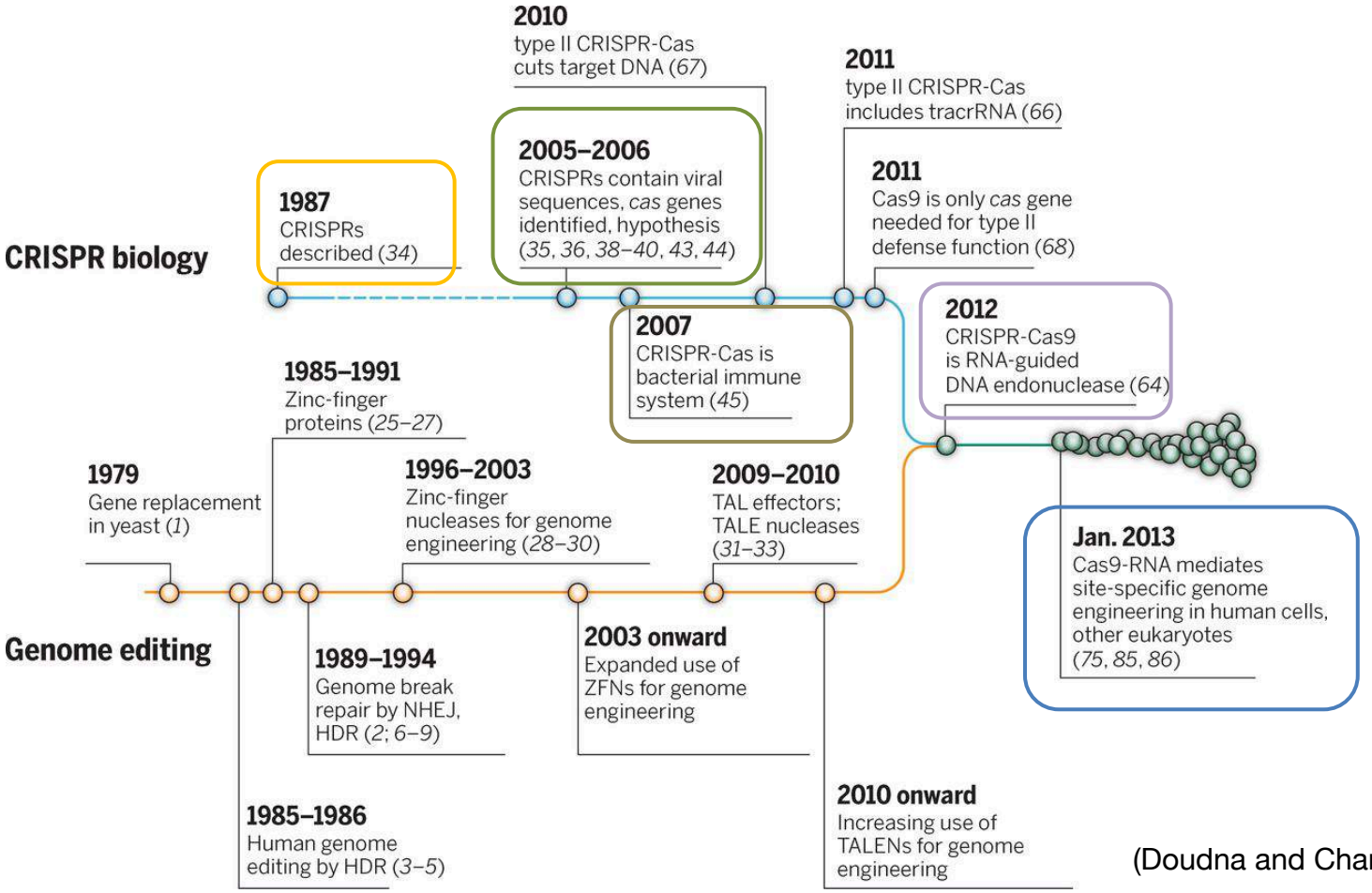

Eugene Koonin


Rodolphe Barrangou


Virginijus Siksnys


Feng Zhang

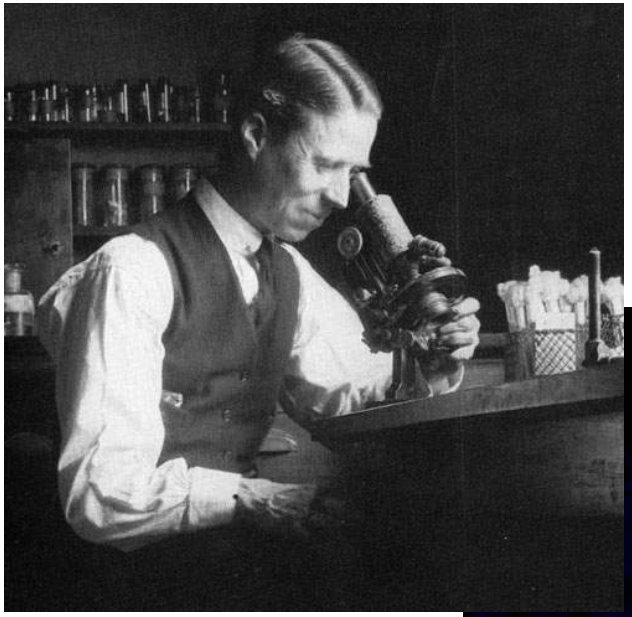

George Church



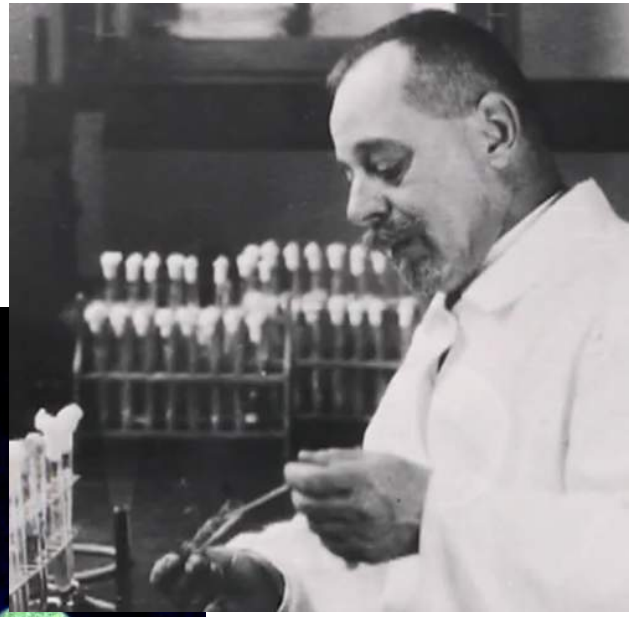
(Doudna and Charpentier, 2014, *Science*)

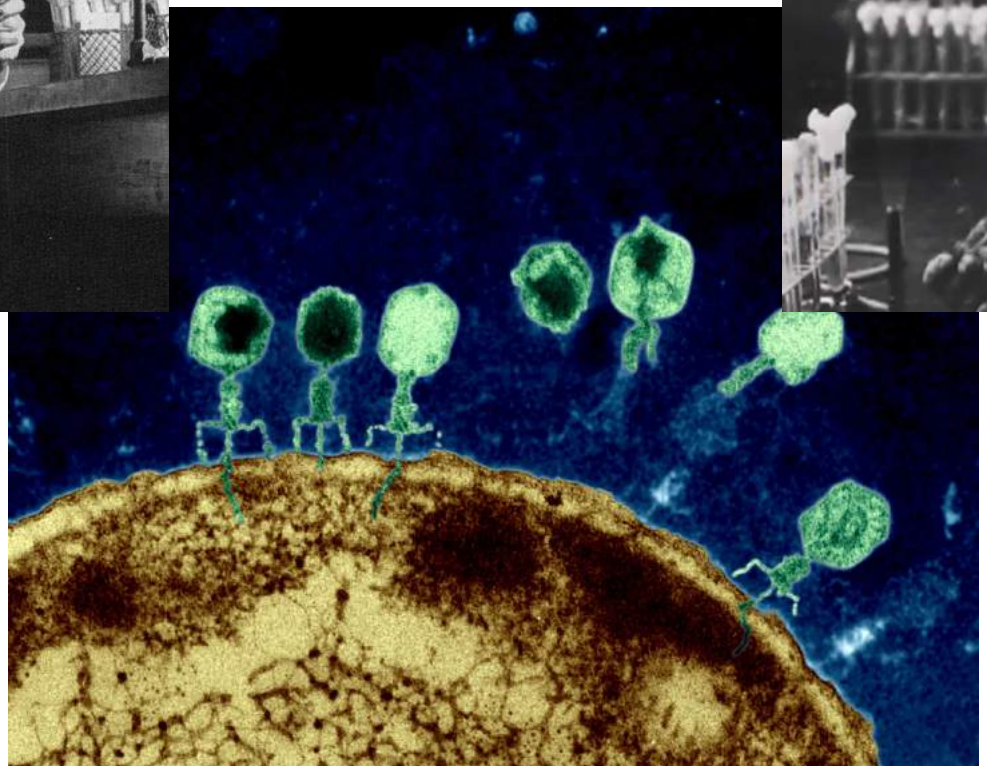
Egy évmilliárdos fegyverkezési verseny

1915 – Frederick Twort:
“Egy tenyészthető enzim”



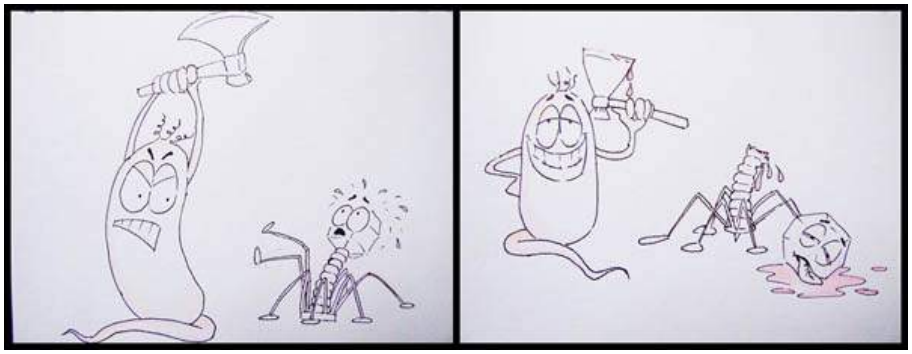
1917 – Felix d’Herelle:
“Bakteriofágok – baktériumok elfogyasztói”



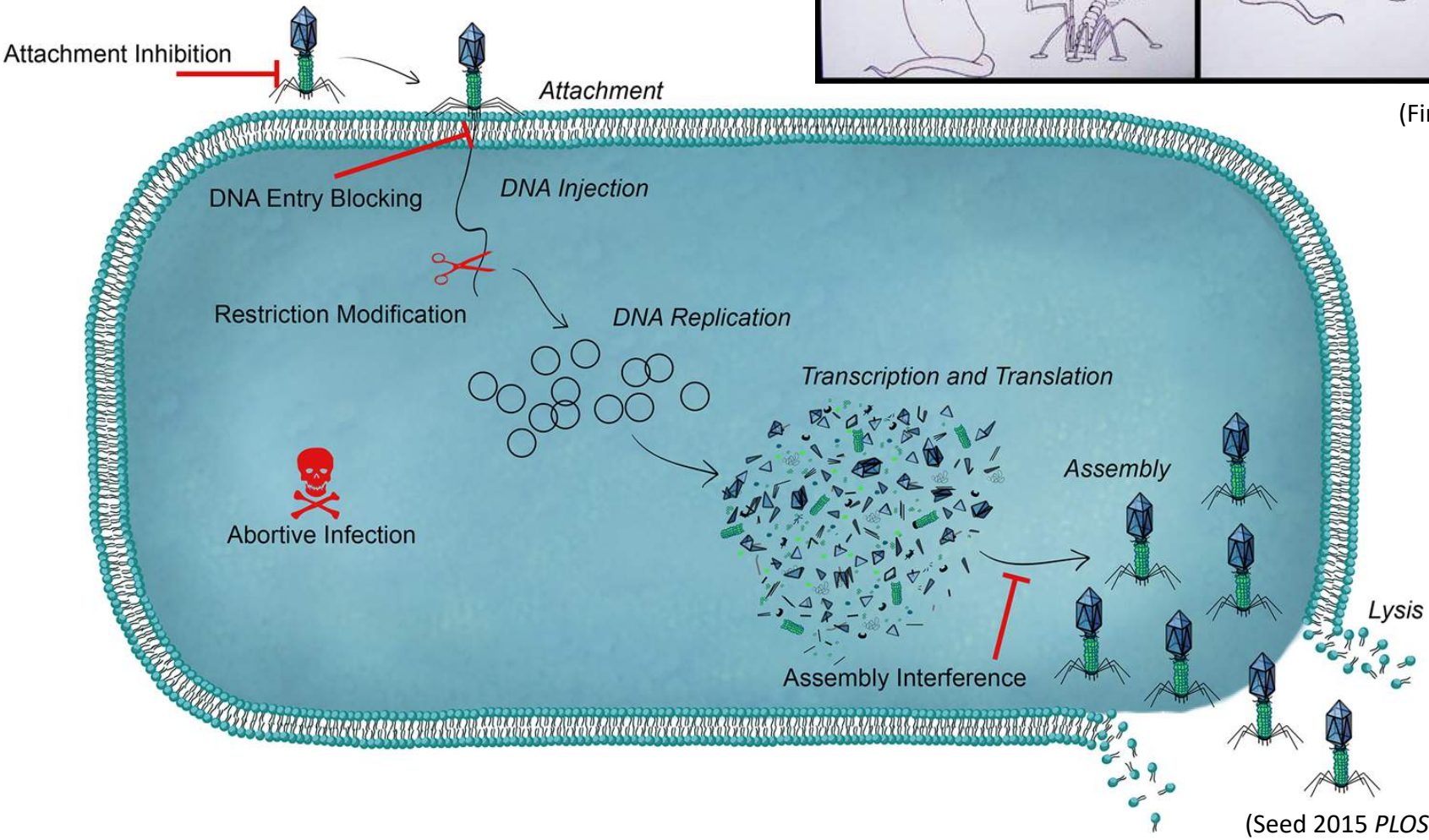


(Fotó: Eye of Science/Science Source)

Fág-ellenes stratégiák baktériumokban

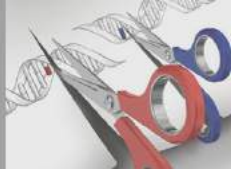


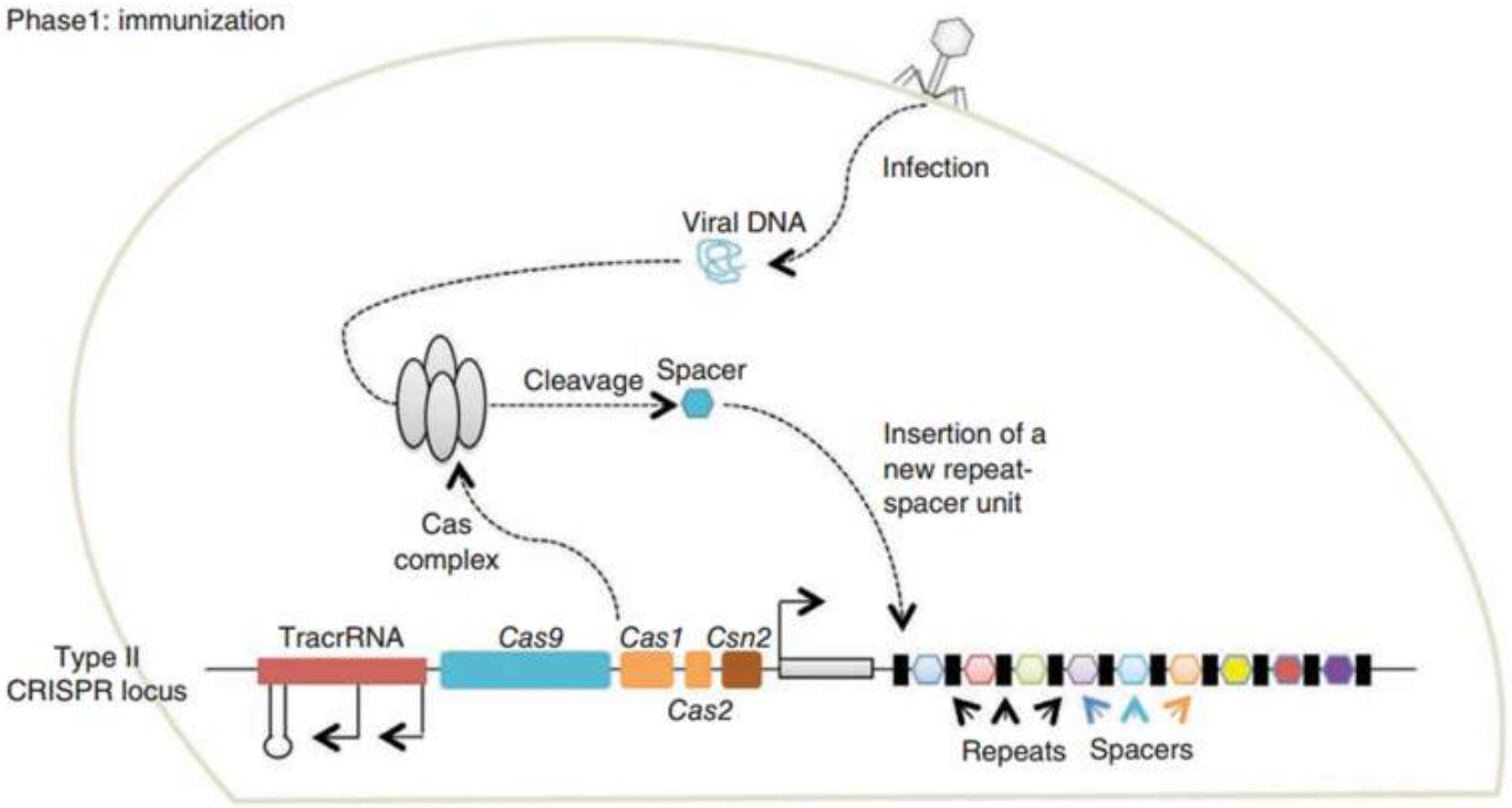
(Fineran labor)



(Seed 2015 *PLOS Pathogens*)

Fág-ellenes stratégiák: prokarióta “immunrendszer”?

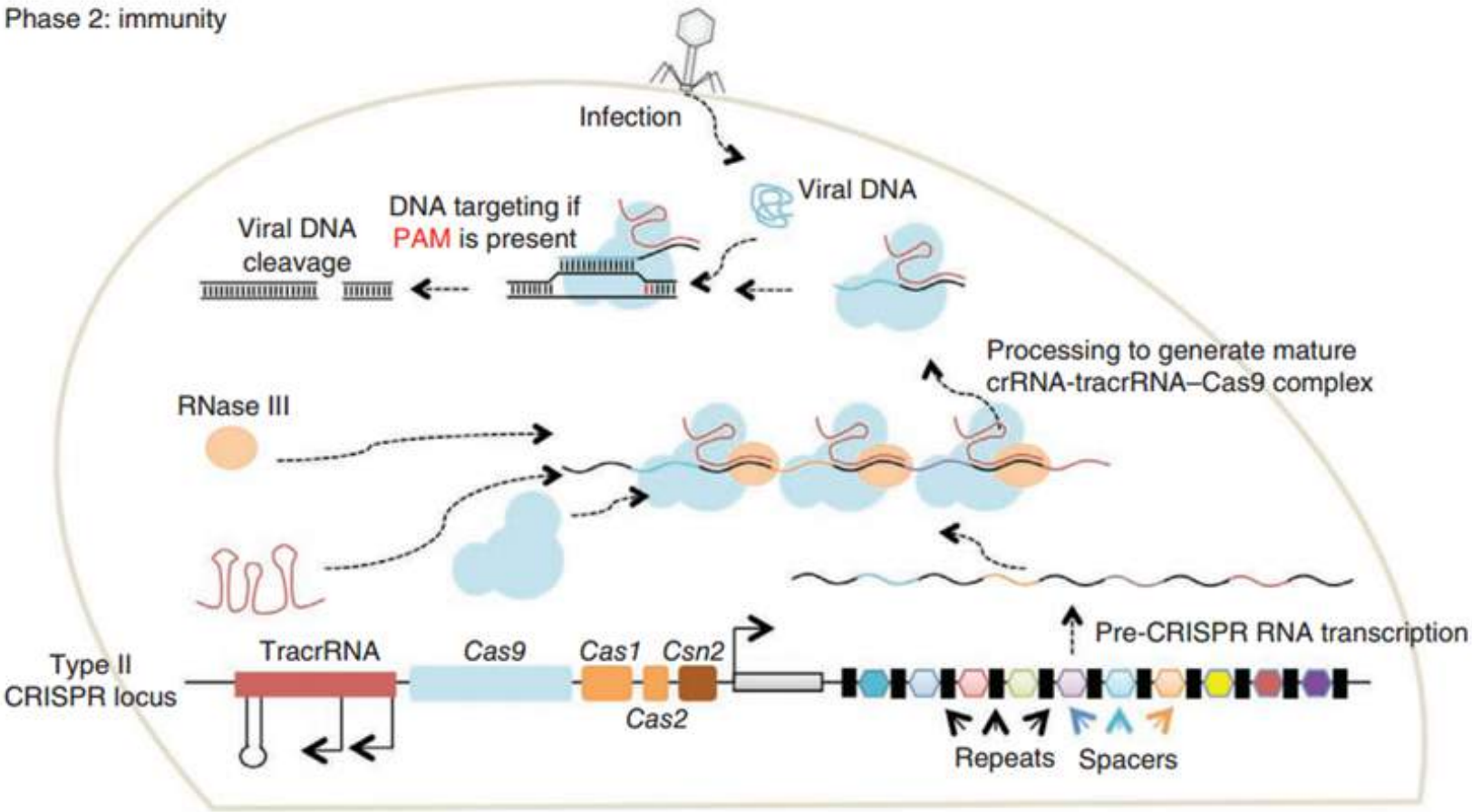




CRISPR = clustered regularly interspaced palindromic repeats

(Mali et al. 2013 *Nat Methods*)

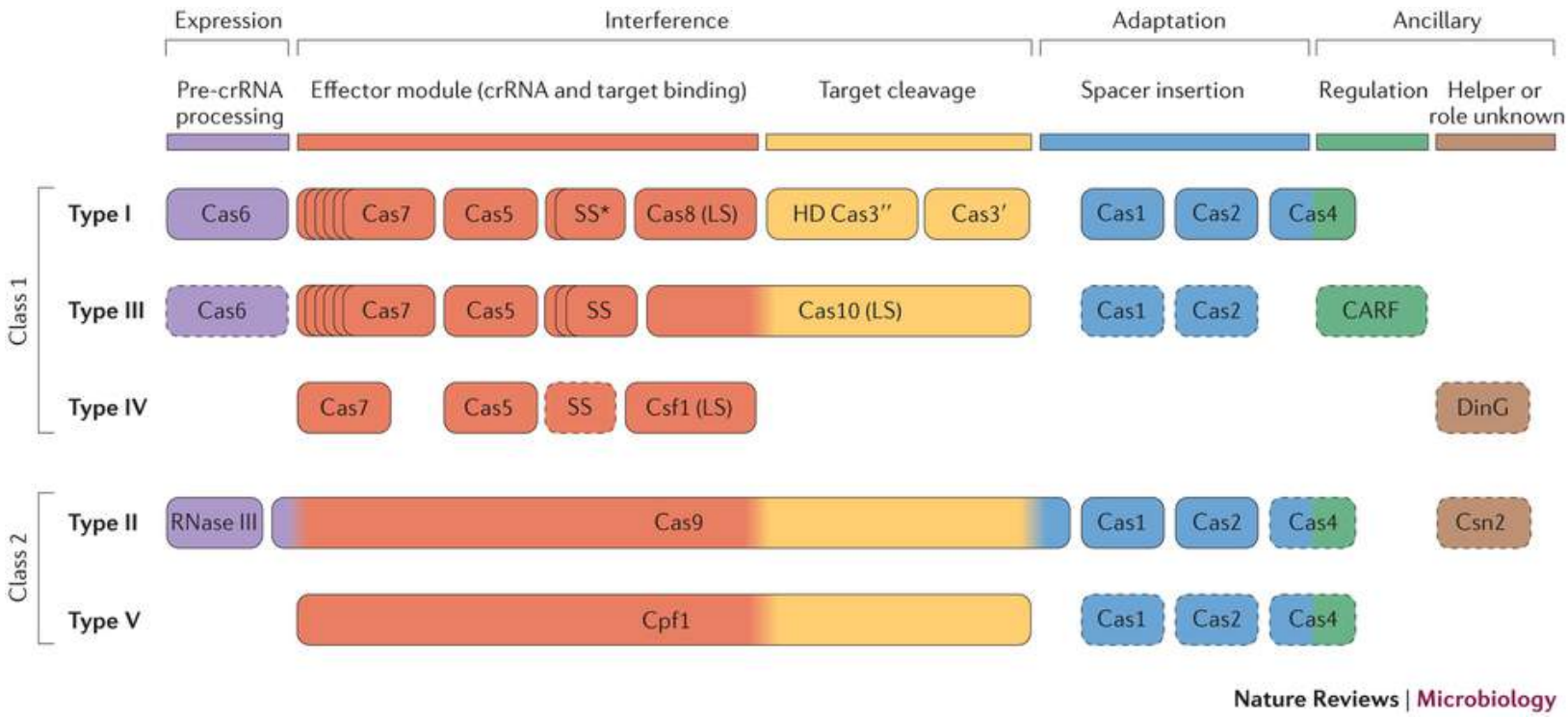
Fág-ellenes stratégiák: prokarióta “immunrendszer”?



CRISPR = clustered regularly interspaced palindromic repeats

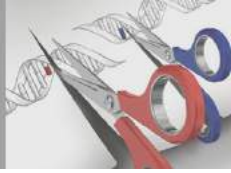
(Mali et al. 2013 *Nat Methods*)

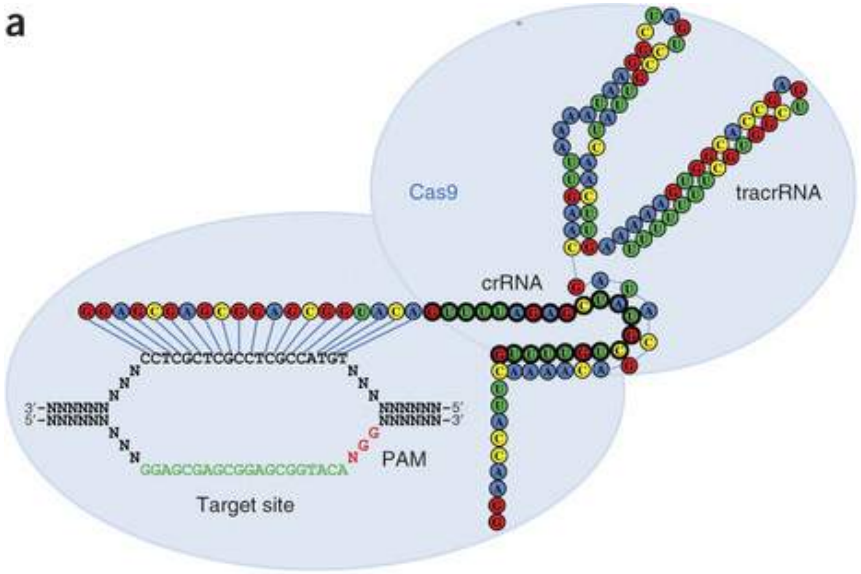
A főbb ismert CRISPR rendszerek



(Makarova et al. 2015 *Nat Rev Micro*)

CRISPR alapú gén-editálás

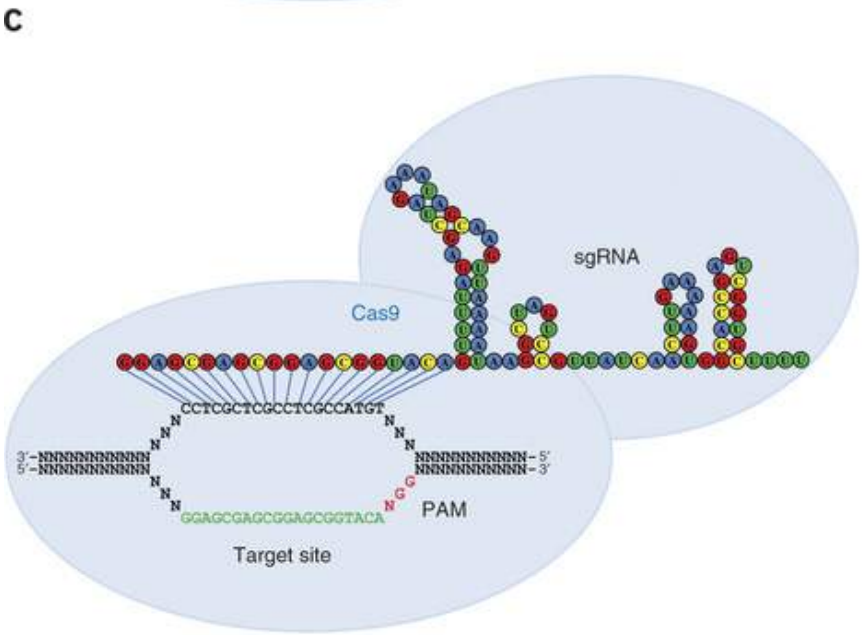


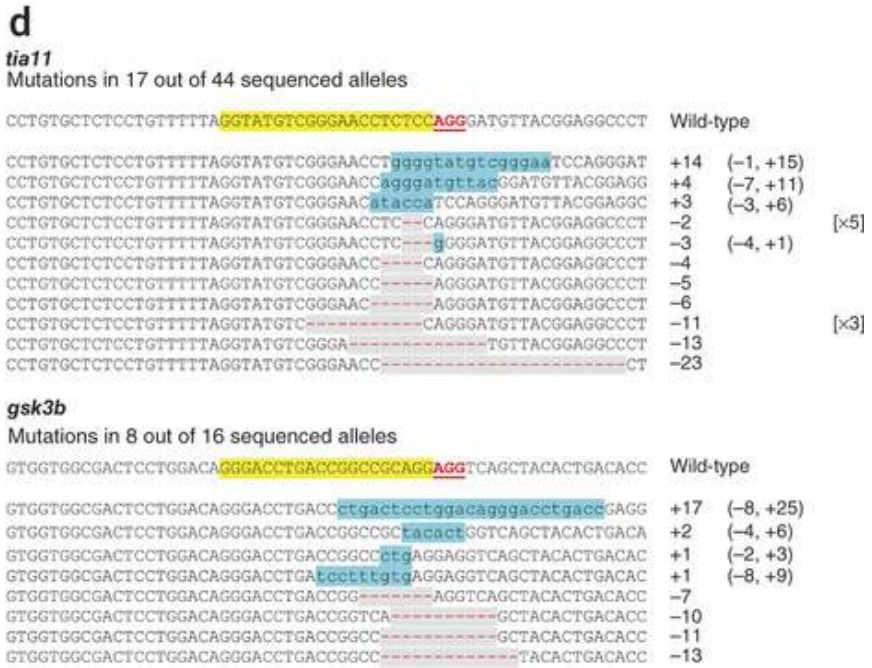


crRNA = CRISPR trageting RNA
tracrRNA = trans activating crRNA

PAM = protospacer adjacent motif

sgRNA = single guide RNA

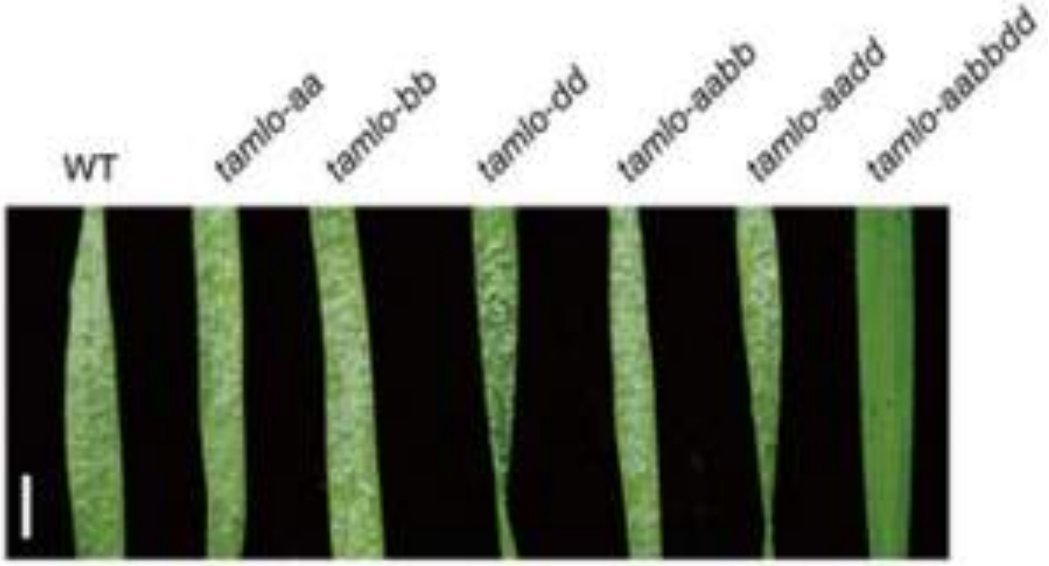




(Hwang et al., 2013, *Nat Biotech*)

Genomszerkesztés növényekben

- a lisztharmatfertőzés feltételezi a működőképes MLO fehérje jelenlétét
- búzában (hexaploid) három pár *MLO* homoeoallél is van, ez nehezíti a védekezést



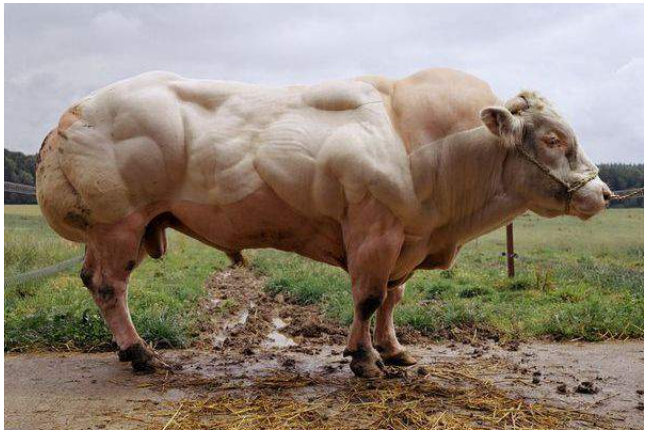
MLO homoeoallélok mutagenezise hexaploid búzában (rezisztencia érhető el így)

(Wang et al. 2014 *Nat Biotech*)

Természetes vs. mesterséges funkcióvesztés: mi a különbég...?

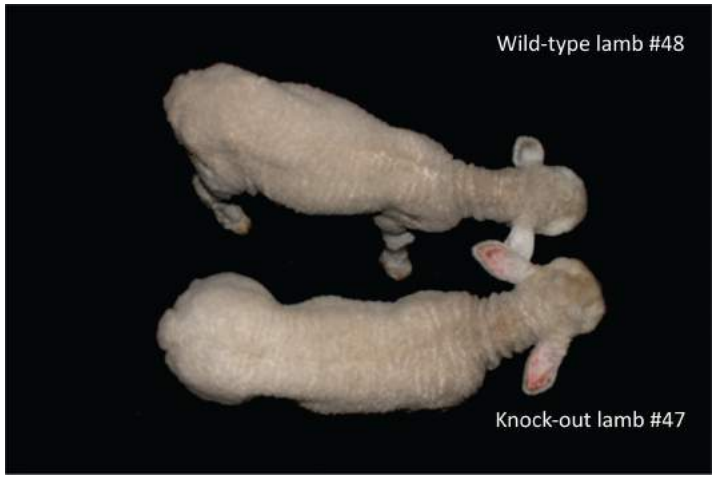
“Texel birka”

“Bully” agár



“Belgian blue”

“Természetes” *MSTN* mutánsok



(Crispo et al 2015 *PLOS One*)

(Zou et al. 2015 *J Mol Cell Biol)*

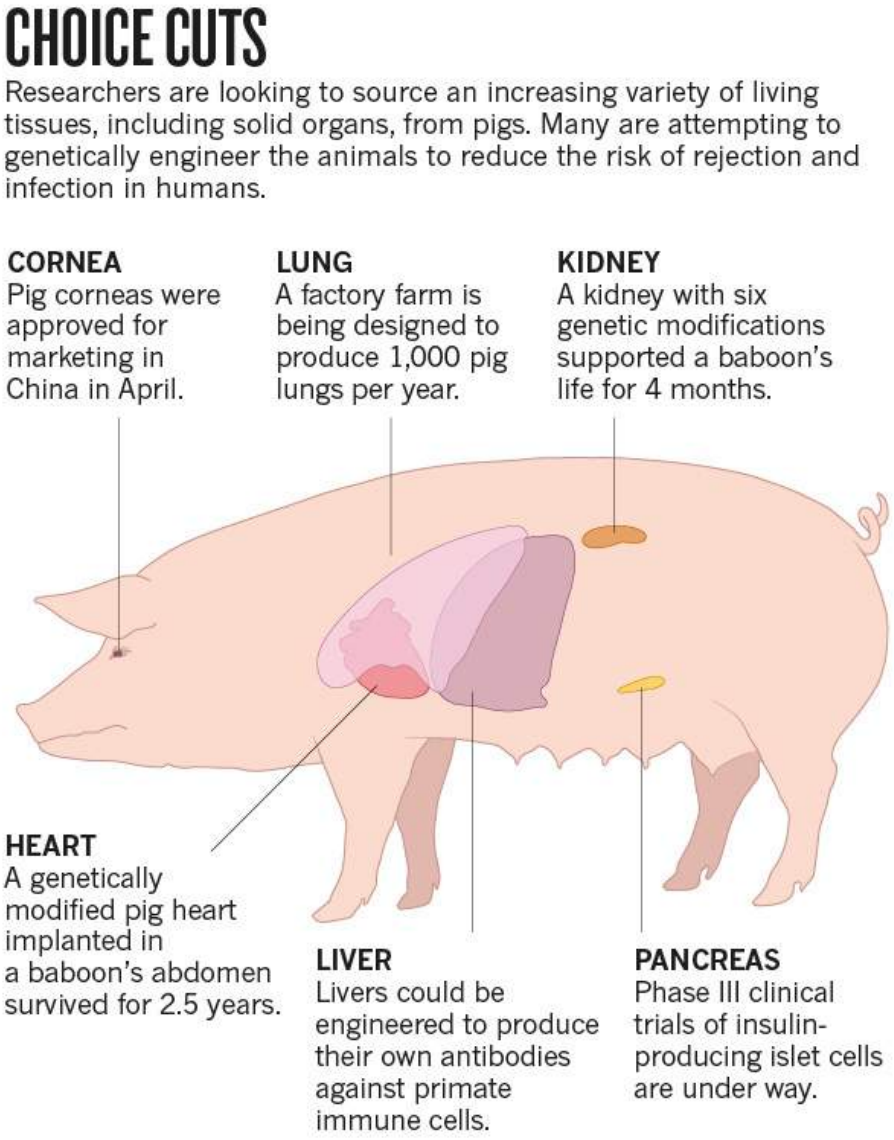
(Wang et al. 2015 *SciRep)*

“Mesterséges” *MSTN* mutánsok

Disznó-eredetű xenotranszplantáció

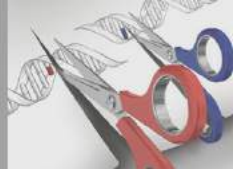
- George Church csoportja képes volt az összes sertés endogén retrovírus (PERV) kópiát inaktiválni – számszerint 62 db-t

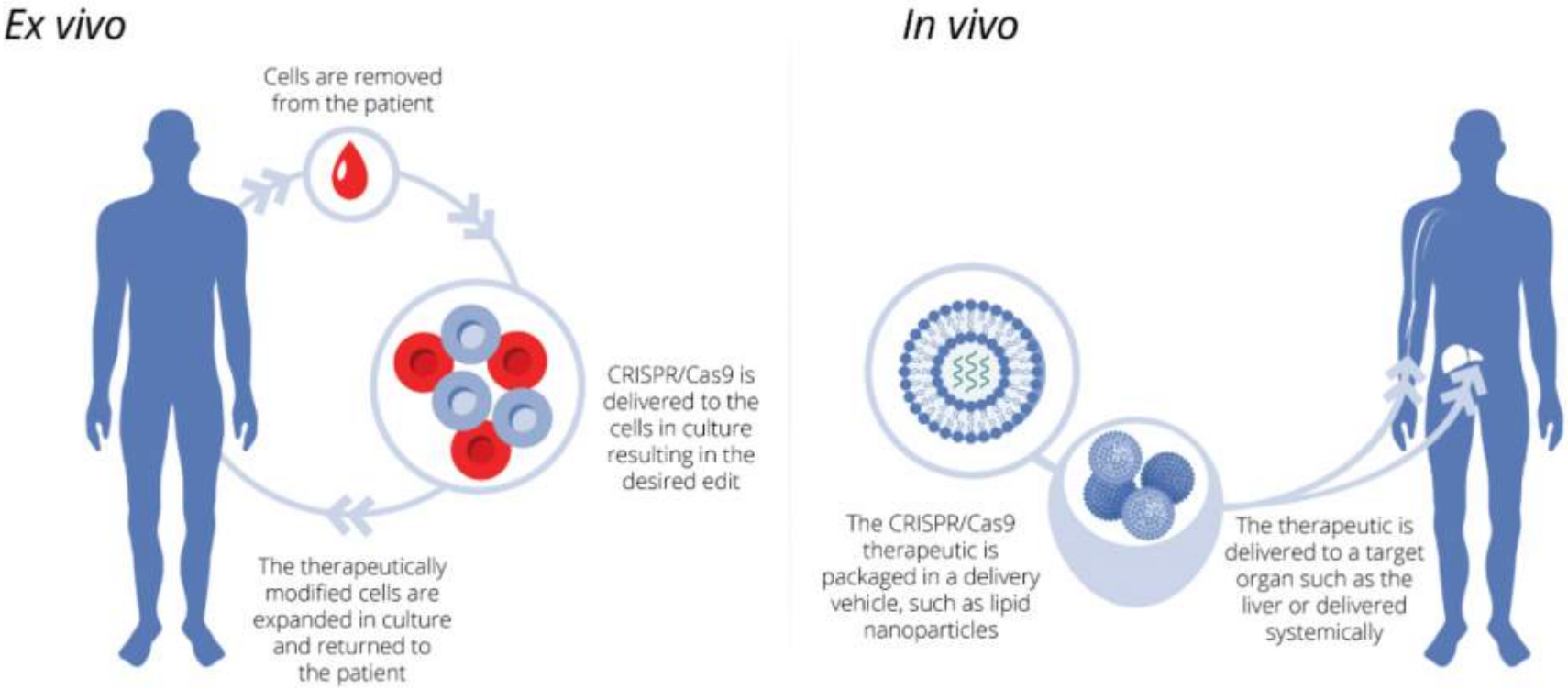
- további 20, sejtfelszíni immunreakciót kiváltó fehérjét kódoló gént is elrontottak



(Reardon 2015 *Nature*)

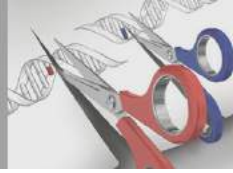
Genomeditálás-alapú terápiák





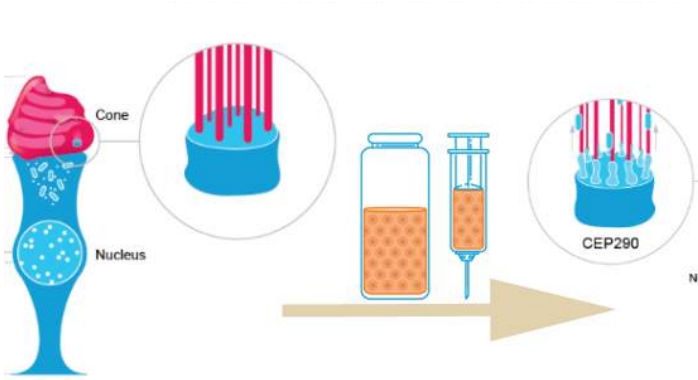
(CRISPR Therapeutics)

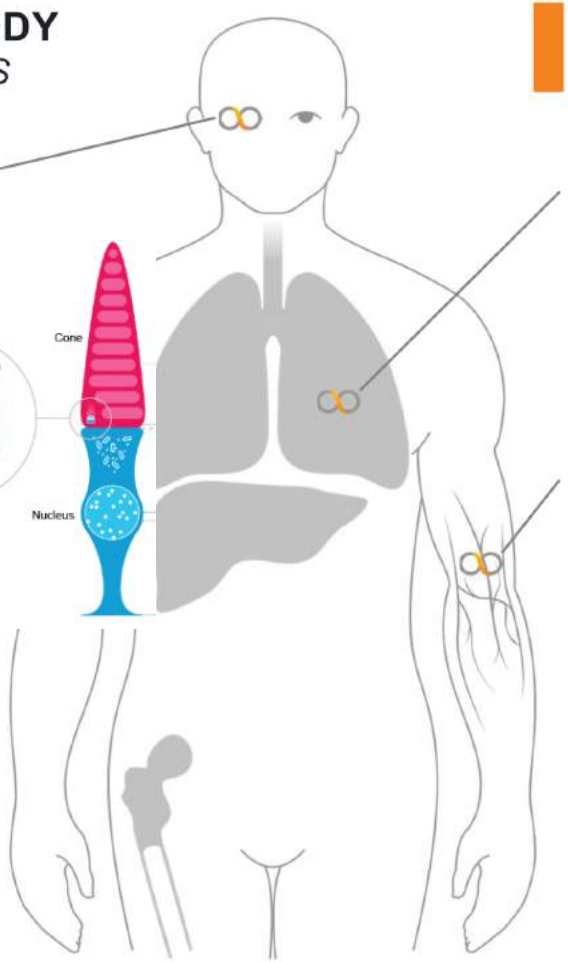
Genomeditálás-alapú terápiák



EDITING INSIDE THE BODY
IN VIVO *CRISPR MEDICINES*

OCULAR DISEASES





EDITING OUTSIDE THE BODY
ENGINEERED CELL MEDICINES

CANCER
Autologous T cell medicines**
Allogeneic cell medicines

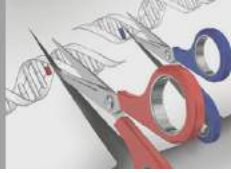
BLOOD DISEASES
Sickle cell disease
Beta-thalassemia

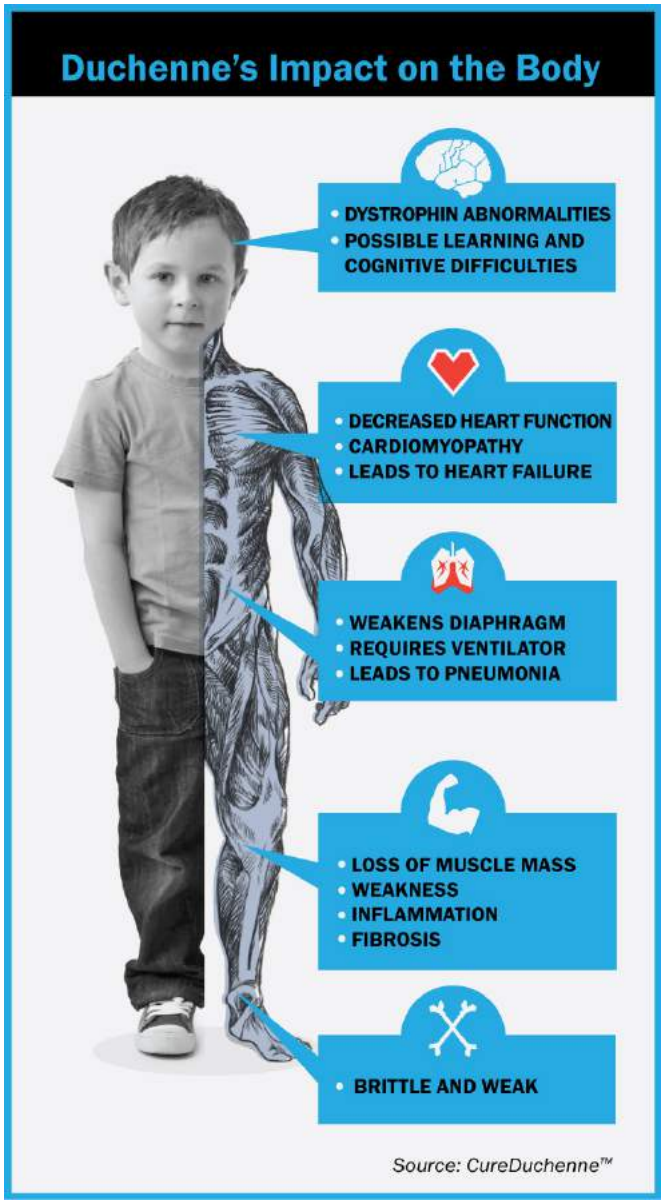
EARLY DISCOVERY
Liver – AATD
Muscle – DMD
Lung – CF

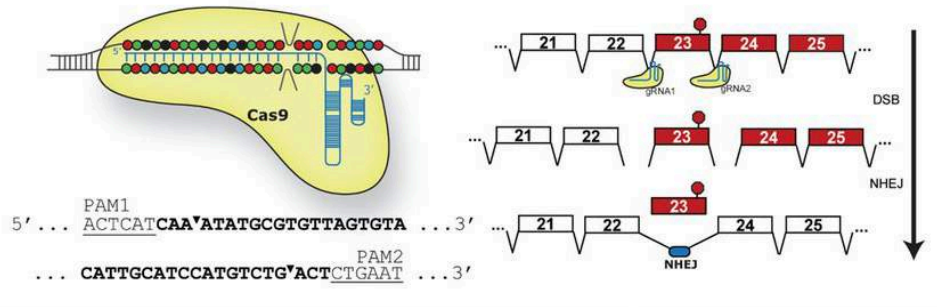
in vitro proof of biology
in vivo proof of concept
AAV delivery

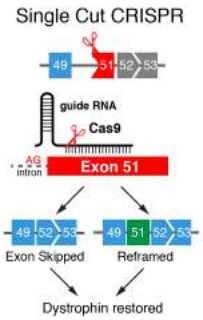
(Editas Medicine)

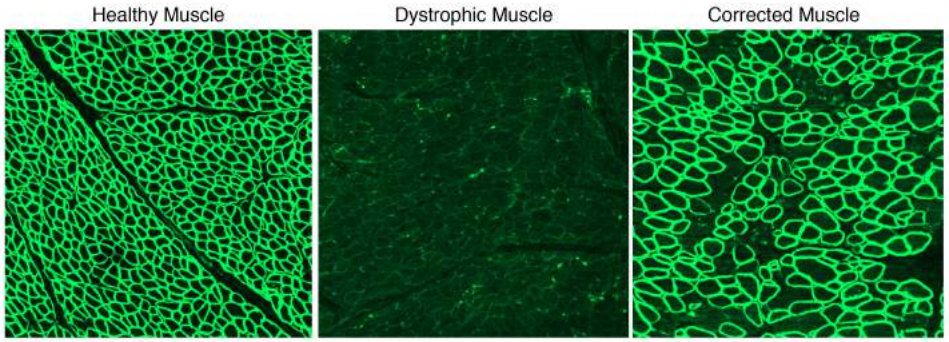
Genomeditálás-alapú terápiák (in vivo)











Kutya – DMD model

(Amoasii et al. 2018 *Science*)

Szép új világot építünk… (már megint)?

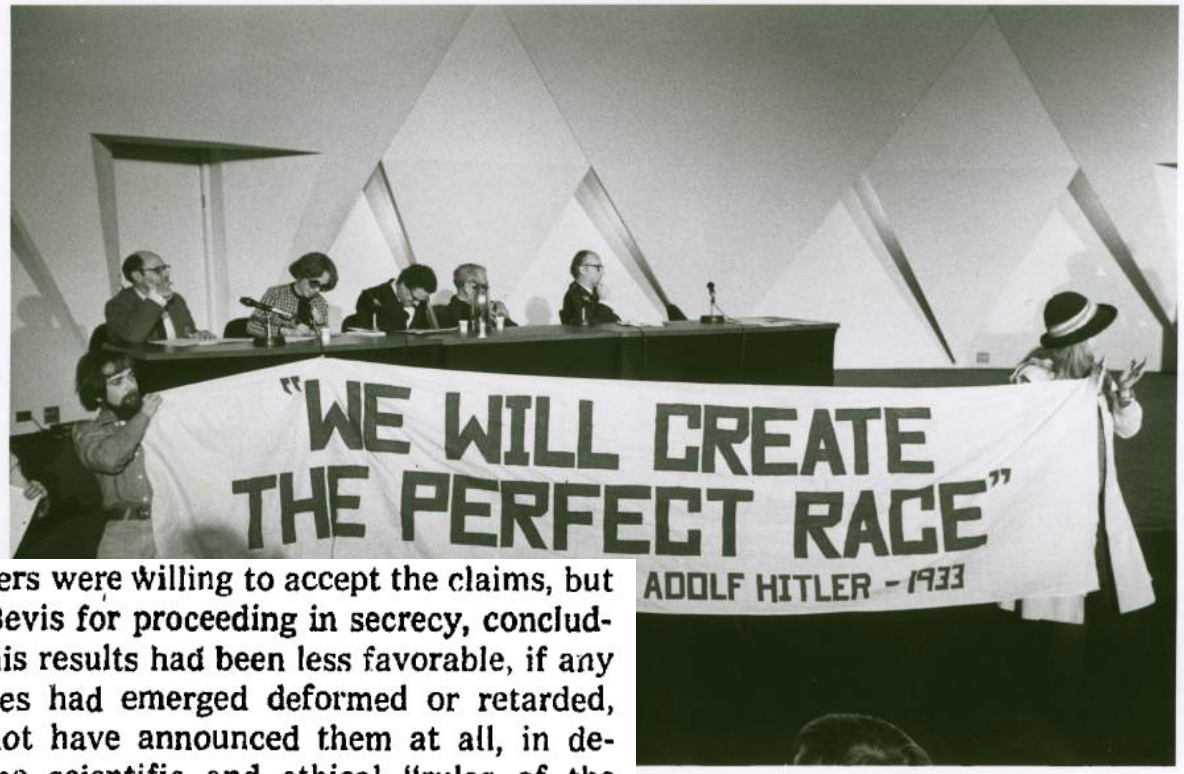
LETTERS

Potential Biohazards of Recombinant DNA Molecules

Recent advances in techniques for the isolation and rejoining of segments of DNA now permit construction of biologically active recombinant DNA molecules in vitro. For example, DNA restriction endonucleases, which generate DNA fragments containing co-

Several groups of scientists are now planning to use this technology to create recombinant DNA's from a variety of other viral, animal, and bacterial sources. Although such experiments are likely to facilitate the solution of important theoreti tical biological problems also result in the creat types of infectious DN whose biological properti completely predicted in

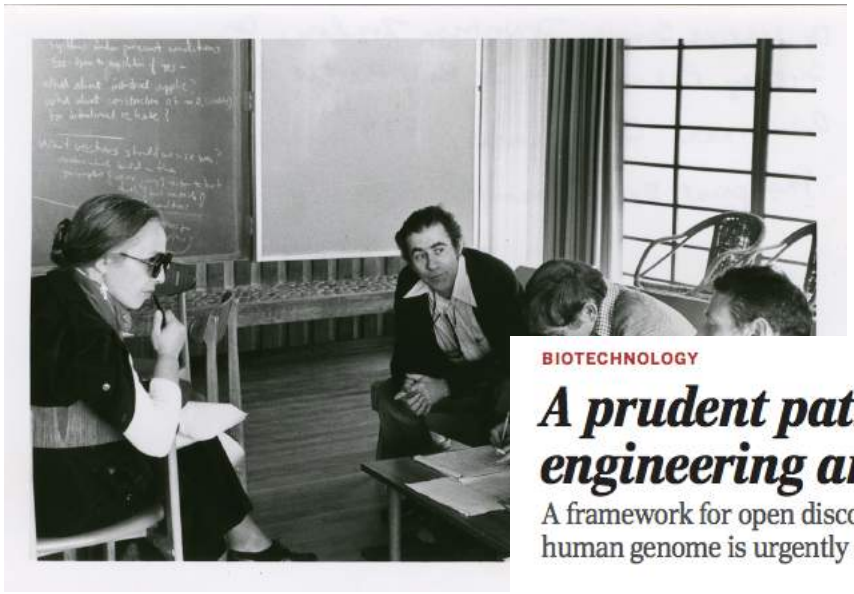
(Berg et al. 1974 *PNAS*)



Some others were willing to accept the claims, but castigated Bevis for proceeding in secrecy, concluding that if his results had been less favorable, if any of the babies had emerged deformed or retarded, he would not have announced them at all, in defiance of the scientific and ethical "rules of the game." Still others overlooked the issue of credibility and, instead, shuddered at the implications of the claims. Not long ago, Dr. James Watson, the Nobel biologist, told a Congressional subcommittee that a successful embryo transplant would soon occur. Watson foresaw "all sorts of bad scenarios" from the achievement. "All hell will break loose, politically and morally, all over the world," he concluded, a bit rashly, some felt.

(15 Sept 1974 *NYTimes*)

Asilomar árnyékában



- 1975 februárjában a kaliforniai Asilomar-ban gyűltek össze kutatók, hogy egy saját szabályrendszert dolgozzanak ki a rekombináns DNS-el való munkára

BIOTECHNOLOGY

A prudent path forward for genomic engineering and germline gene modification

A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed

By David Baltimore,[1] Paul Berg,[2] Michael Botchan,[3,4] Dana Carroll,[5] R. Alta Charo,[6] George Church,[7] Jacob E. Corn,[4] George Q. Daley,[8,9] Jennifer A. Doudna,[4,10]* Marsha Fenner,[4] Henry T. Greely,[11] Martin Jinek,[12] G. Steven Martin,[13] Edward Penhoet,[14] Jennifer Puck,[15] Samuel H. Sternberg,[16] Jonathan S. Weissman,[8,17] Keith R. Yamamoto[4,18]

Genome engineering technology offers unparalleled potential for modifying human and nonhuman genomes. In humans, it holds the promise of curing genetic disease, while in other organisms it provides methods to reshape the biosphere for the benefit of the environment and human societies. However,

- 2015 január: a kaliforniai Napa-ban Asilomar-redux, ezúttal a CRISPR apropóján

#geneeditsummit

A dizájner bébik hajnala?

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

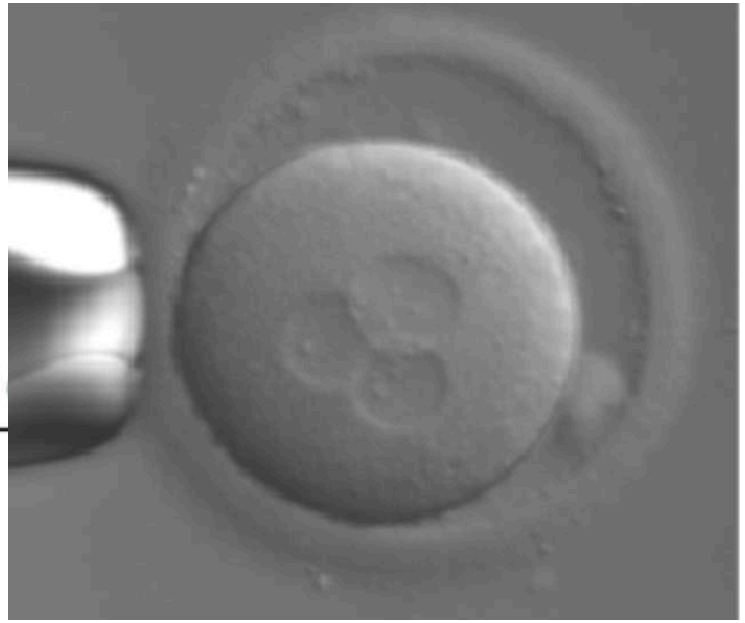
Guangdong Province Key Laboratory of Reproductive Medicine, the First Affiliated Hospital, and Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China

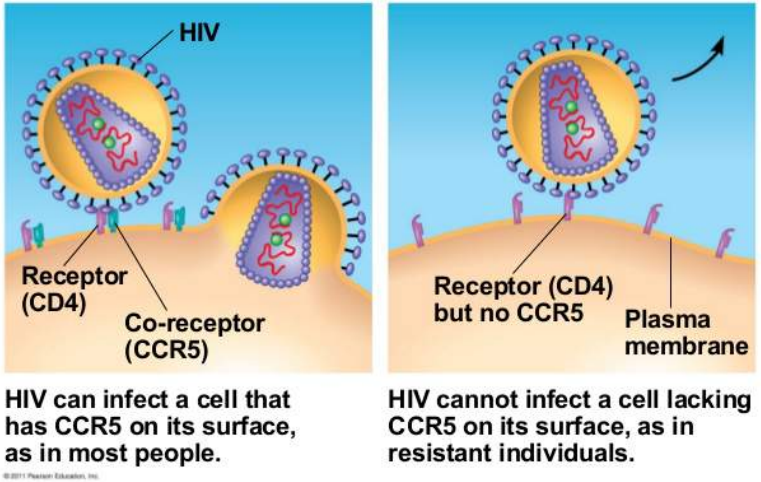
J Assist Reprod Genet
DOI 10.1007/s10815-016-0710-8

TECHNOLOGICAL INNOVATIONS

Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing

Xiangjin Kang[1] · Wenyin He[1] · Yuling Huang[1] · Qian Yu[1] · Yaoyong Chen[1] · Xingcheng Gao[1] · Xiaofang Sun[1] · Yong Fan[1]



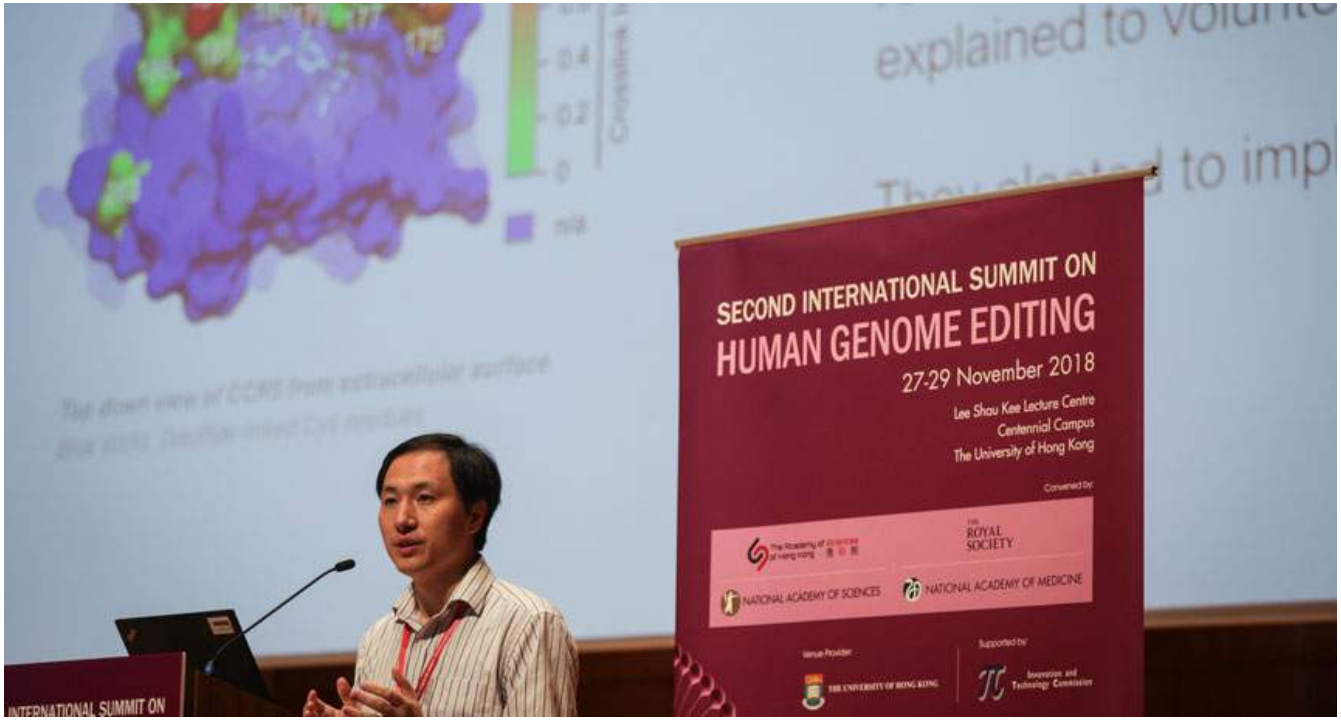


HIV can infect a cell that has CCR5 on its surface, as in most people.

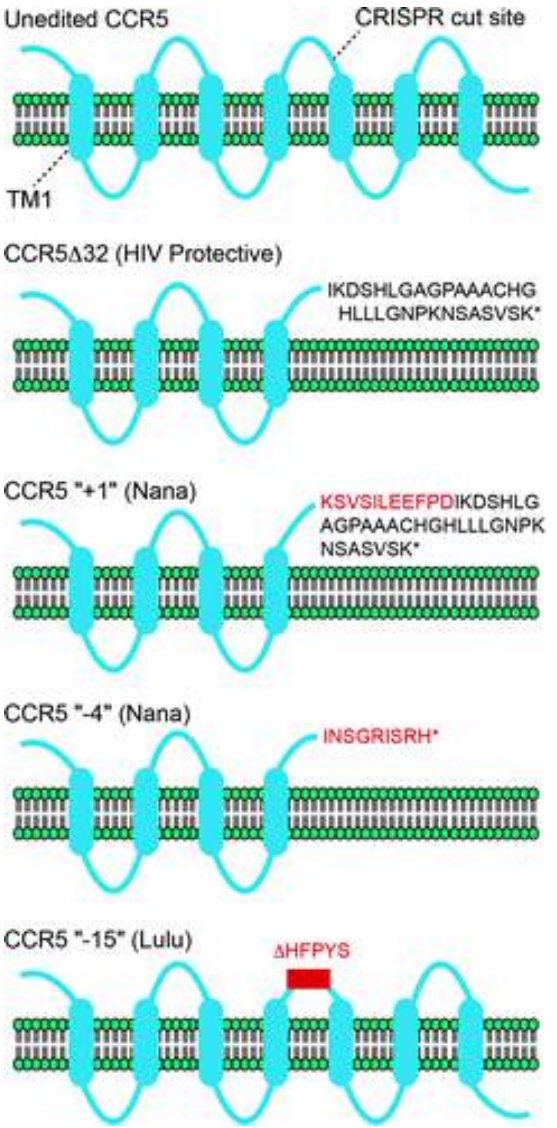
HIV cannot infect a cell lacking CCR5 on its surface, as in resistant individuals.

(Liang et al. 2015 *Protein&Cell,* Kang et al. 2016 *J Assist Reprod Genetl*)

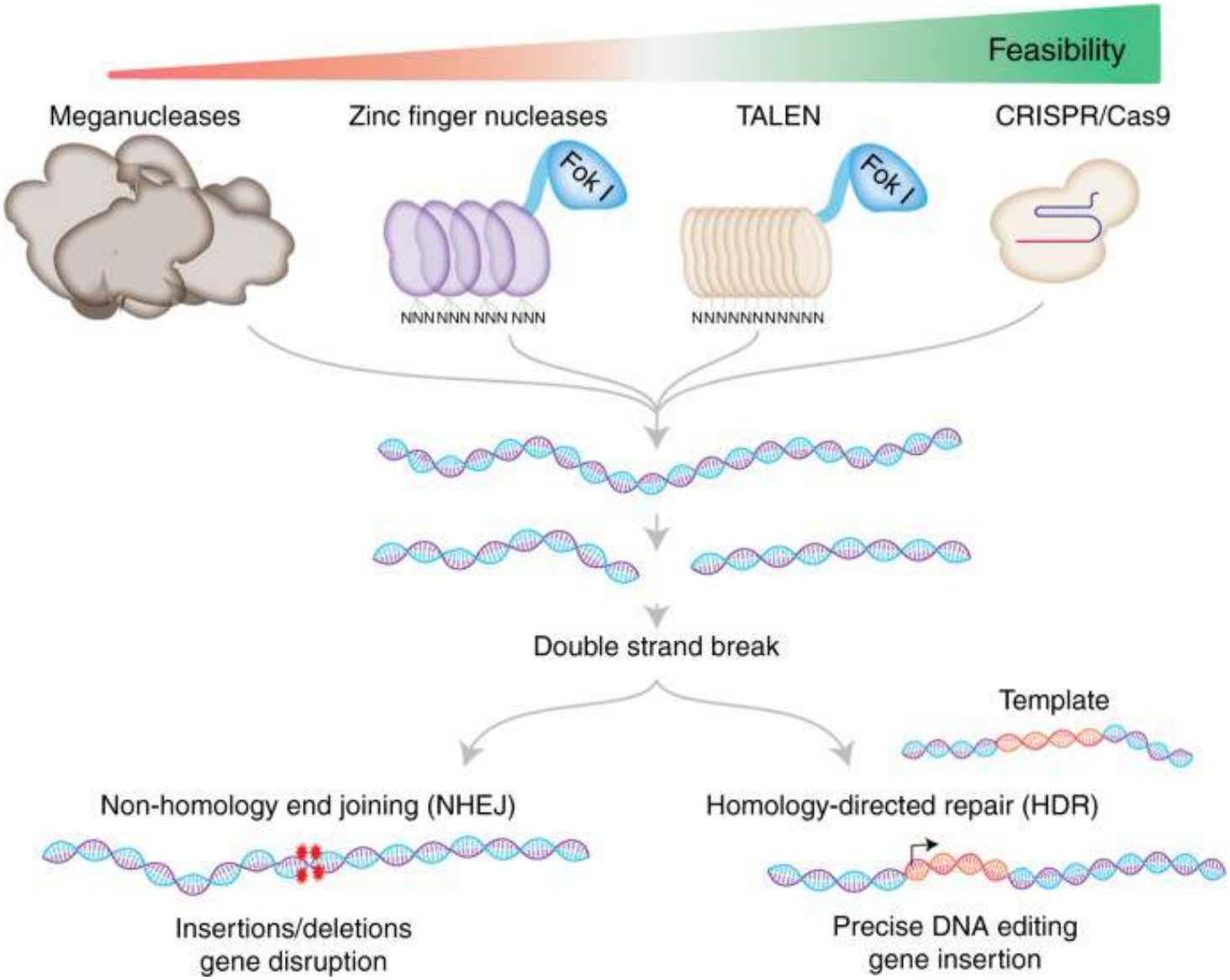
A dizájner bébik hajnala?



(Csiankuj Ho – 2018 november)

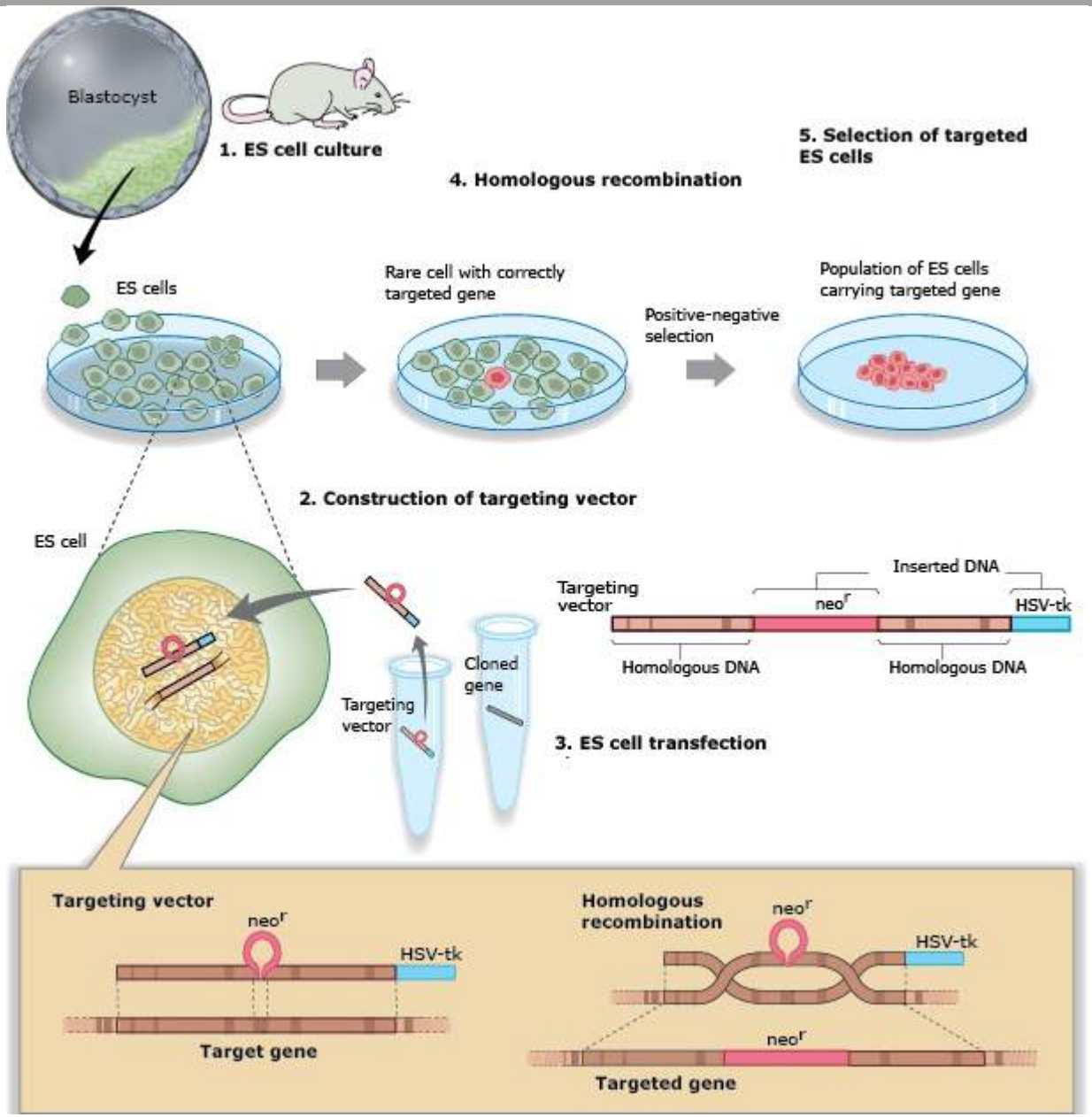


Az igazi „szerkesztés”: homológ rekombináció



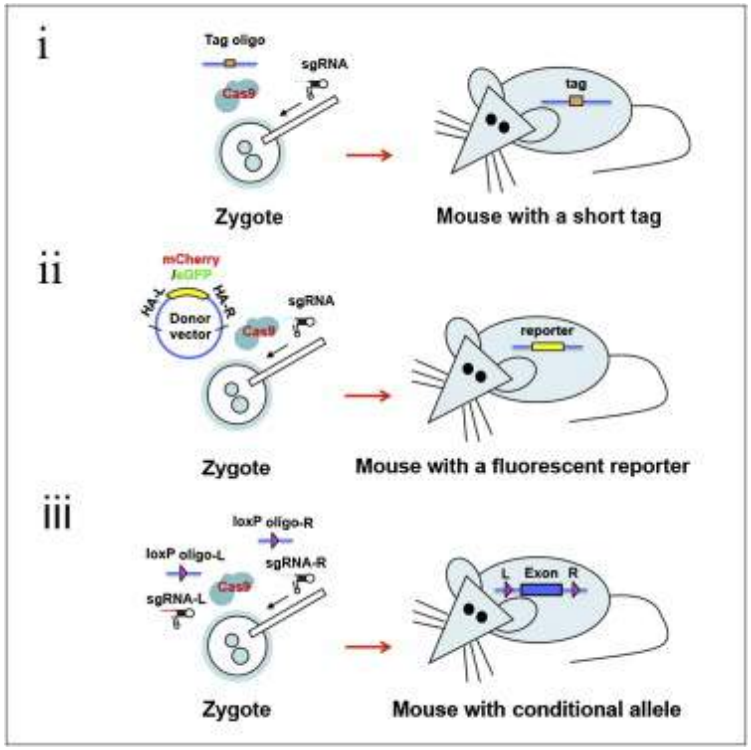
(Wikipedia)

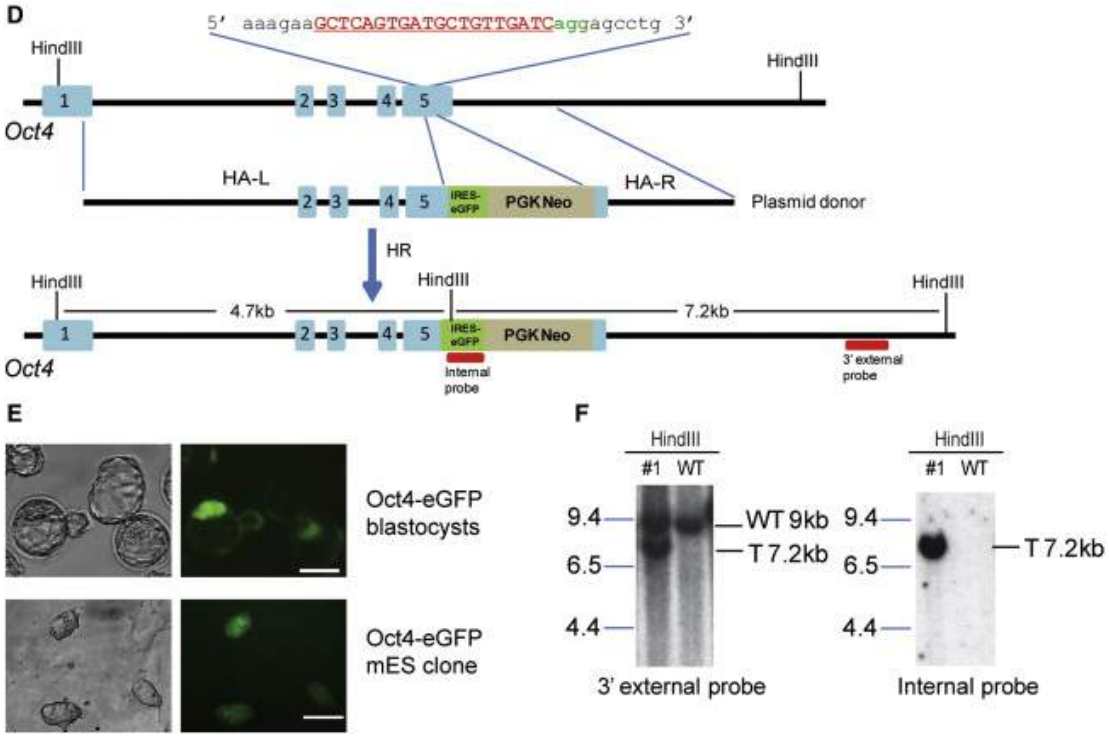
Géneditálás homológ rekombinációval (HR)



- klasszikus HR: 1:10 000 embrió (!!)
- I-SceI emésztés: 50x hatékonyabb
- “klasszikus” Cas9 (mRNS inj): 1:8, Cas9RNP: 1:2

Knock-in egerek készítése CRISPR segítségével





(Yang et al., 2013, *Cell*)

Cold Spring Harbor Laboratory

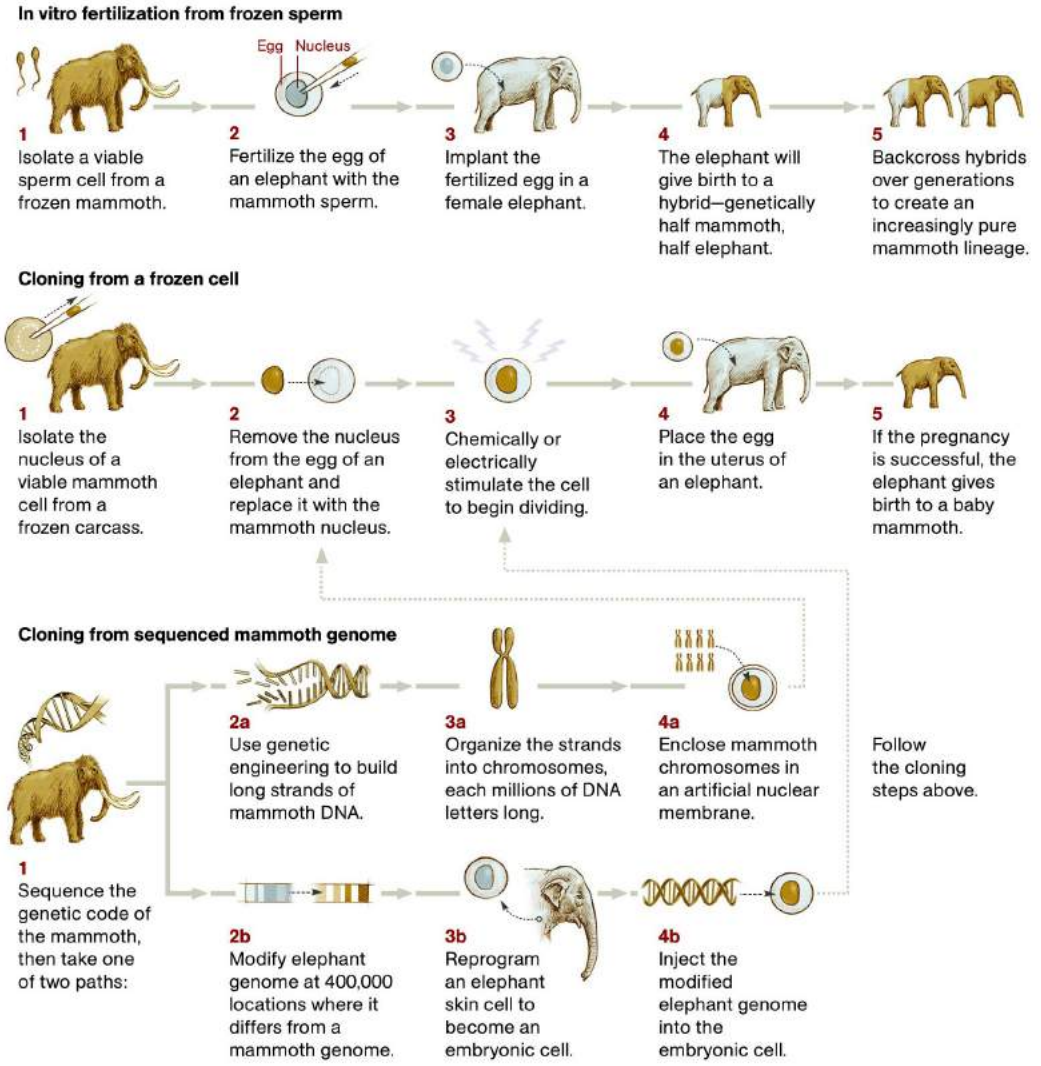
bioRχiv
THE PREPRINT SERVER FOR BIOLOGY

doi: 10.1101/393231

Re-Evaluating One-step Generation of Mice Carrying Conditional Alleles by CRISPR-Cas9-Mediated Genome Editing Technology

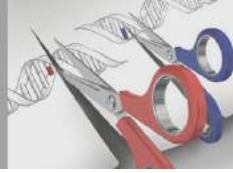
The dataset constituted 17,887 microinjected or electroporated zygotes and 1,718 live born mice, of which only 15 (0.87%) mice harbored 2 correct LoxP insertions in cis configuration indicating a very low efficiency of the method.

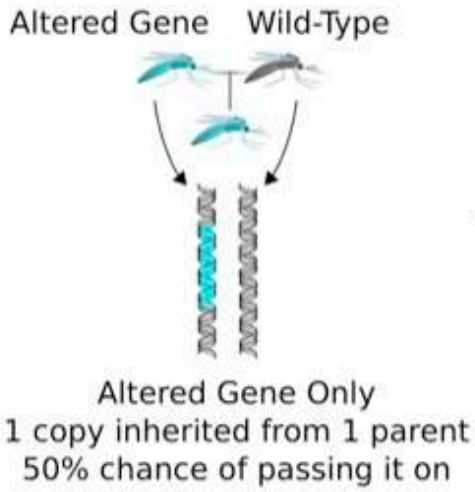
De-extinkció: kihalt fajok “újraélesztése”

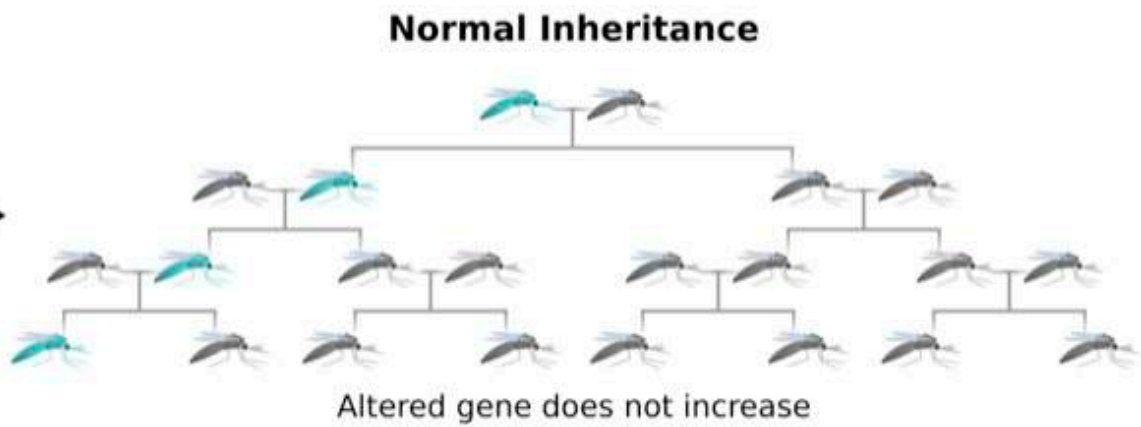


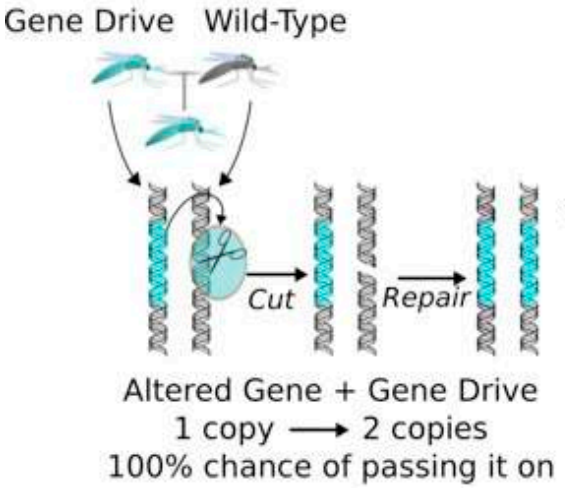
(National Geographic)

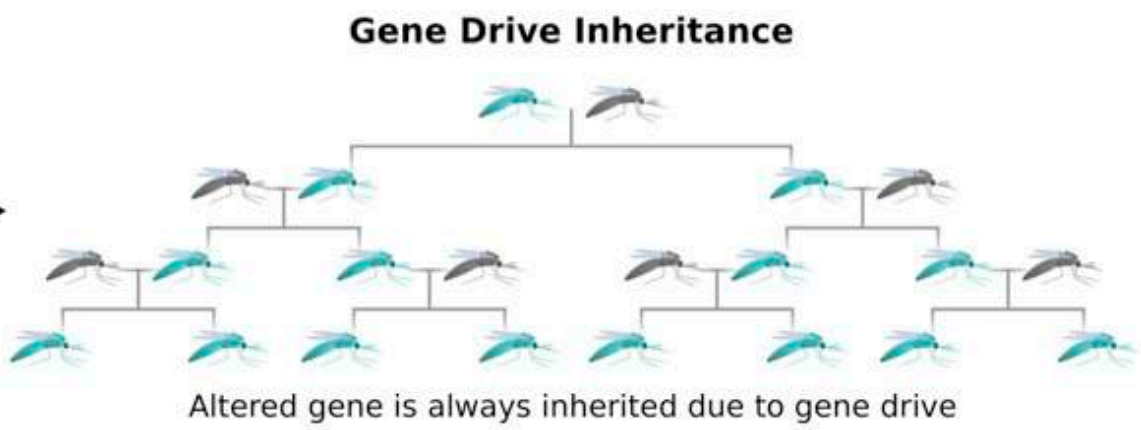
“Gene drive” – ökológiai mumus, vagy csodaszer





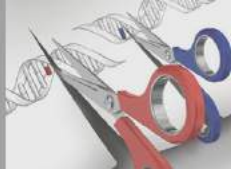






(Scientific American)

További olvasnivaló



WIRED

The Battle Over Genome Editing Gets Science All Wrong
http://www.wired.com/2015/10/battle-genome-editing-gets-science-wrong/

THE NEW YORKER

The Gene Hackers
http://www.newyorker.com/magazine/2015/11/16/the-gene-hackers

nature

CRISPR, the disruptor
http://www.nature.com/news/crispr-the-disruptor-1.17673

EMBO reports

No time to waste—the ethical challenges created by CRISPR
http://embor.embopress.org/content/16/11/1421.long

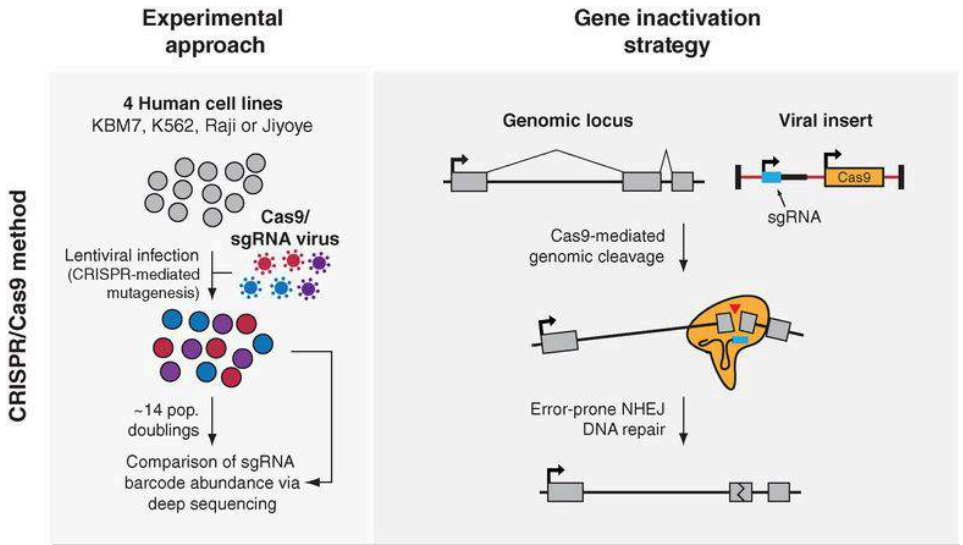
SCIENTIFIC AMERICAN™

"Gene Drives" And CRISPR Could Revolutionize Ecosystem Management
http://blogs.scientificamerican.com/guest-blog/gene-drives-and-crispr-could-revolutionize-ecosystem-management/

STAT

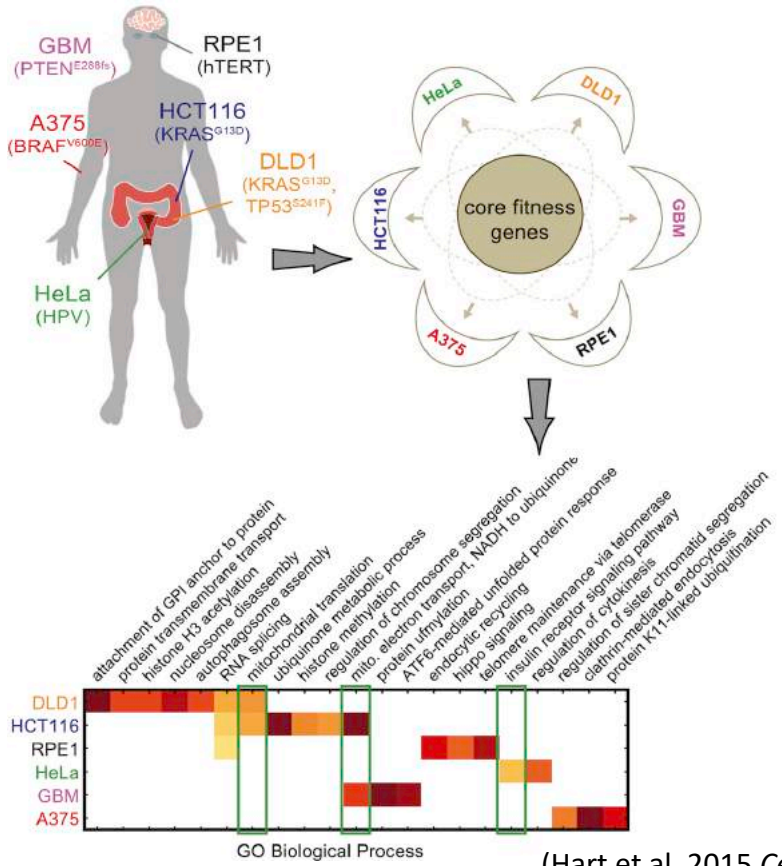
A debate: Should we edit the human genome?
http://www.statnews.com/2015/11/30/gene-editing-crispr-germline/

Esszenciális gének normál vs. rákos sejtekben



(Wang et al. 2015 *Science*)

- a fehérje kódoló gének kb. 10%-a esszenciális (~2000 gén) az emberi *sejtvonalak* in vitro fennmaradásához



- különböző rákos sejtvonalak vizsgálata megmutathatja, hol van az egyes betegségek Achilles-ina, hol lehet olyan célzott terápiát alkalmazni, ami specifikusan a daganatos sejteket támadja

(Hart et al. 2015 *Cell*)