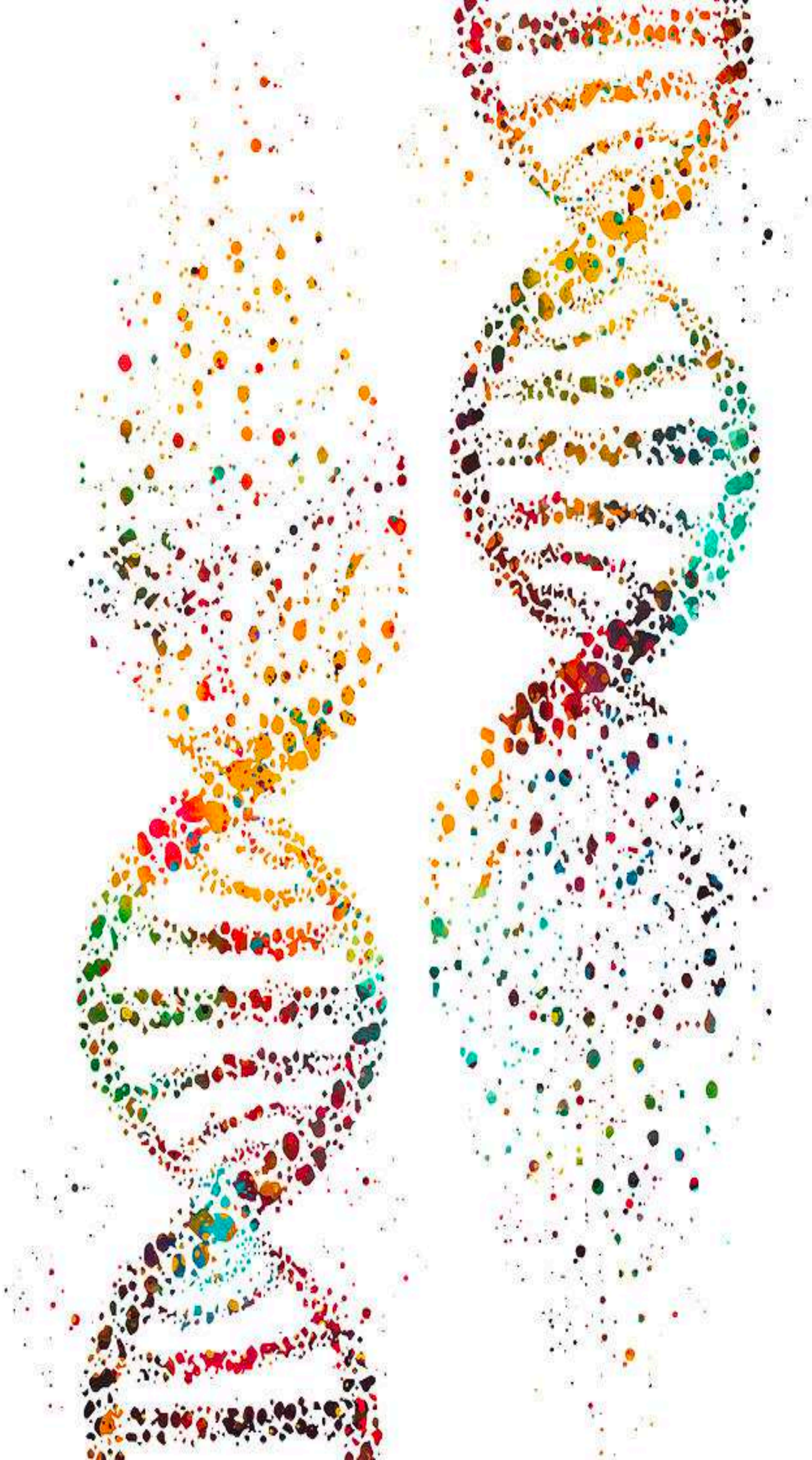


Harder, Better, Faster, CRISPR:

The Past, Present, and Future of Genetic Engineering

Steve Knutson

May 24, 2022

October 7, 2020

Emmanuelle Charpentier
Max Planck Institute for Infection biology, Berlin

Jennifer Doudna
University of California, Berkeley

- > 10,000 research articles
- 45 completed and ongoing clinical trials
- >70 active NIH R01s
- $17B valuation across >12 companies
- 6-year, >$100M patent lawsuit
- 1 person in prison

Early genetic engineering - selective breeding

Early genetic engineering - selective breeding

Early genetic engineering - selective breeding

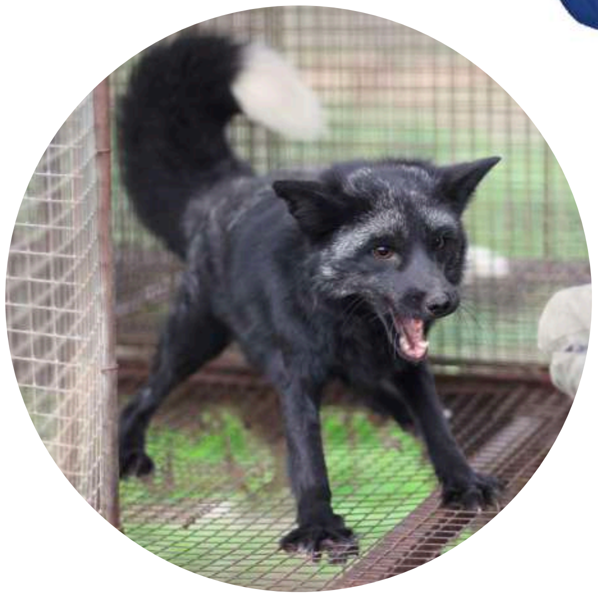
Early genetic engineering - selective breeding

The Siberian fox experiment

silver fox
Vulpes vulpes

Dmitry Belyayev

The Siberian fox experiment



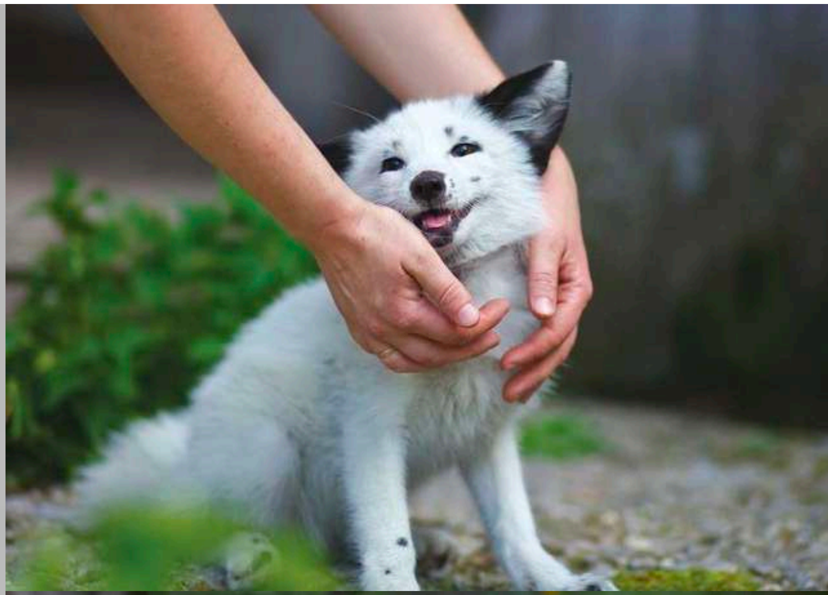
fear, aggression, violence

do not breed

indifference, curiousity

breed

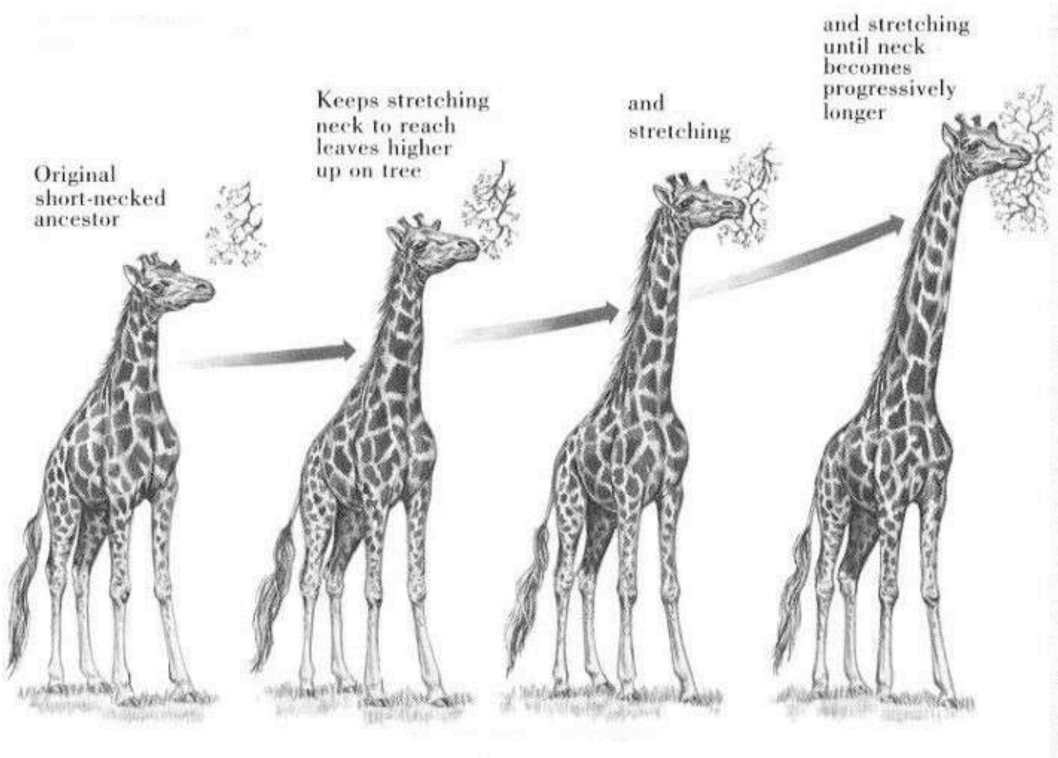
The Siberian fox experiment

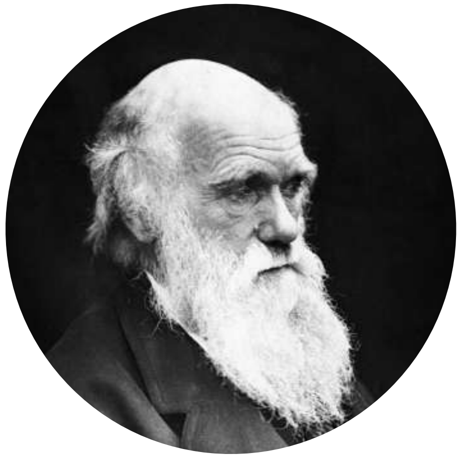


Genetics can be… complicated

Jean-Baptiste Lamarck - 1770s

- *physical changes during life are heritable*
- *these are passed on to future generations*





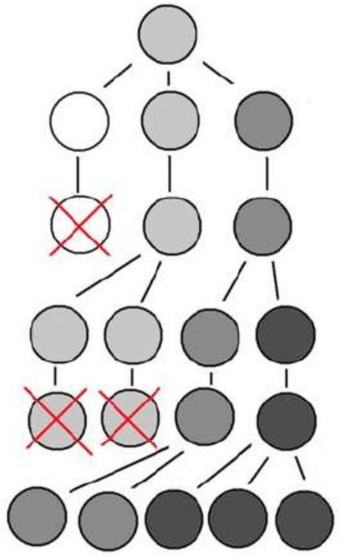
Charles Darwin - 1859

- *variation in traits exist across generation*
- *most "successful" variants reproduce*

peppered moth, UK

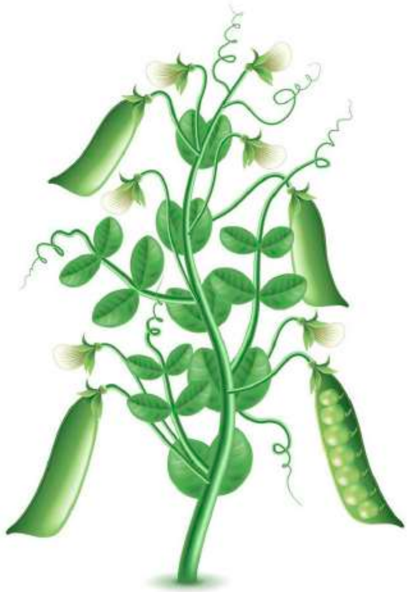
pre 1850s

after 1900



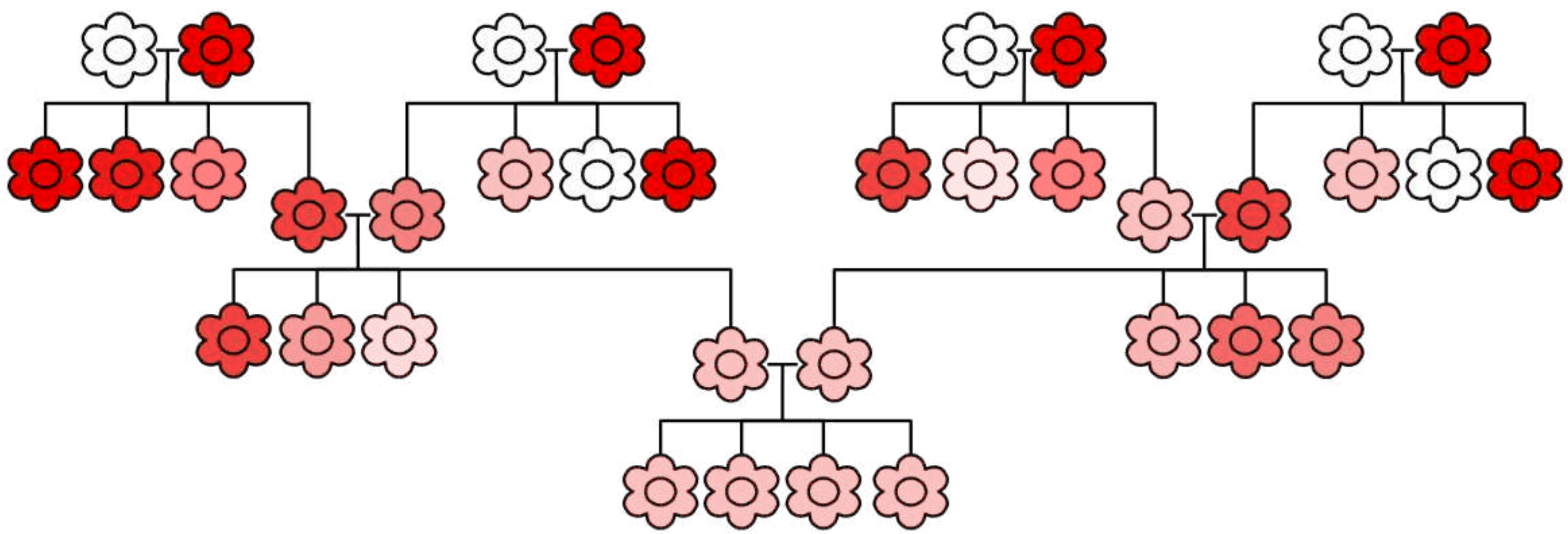
Genetics can be… complicated

Gregor Mendel - 1863



pea plant
pisum sativum

"blending" inheritance - is it true?



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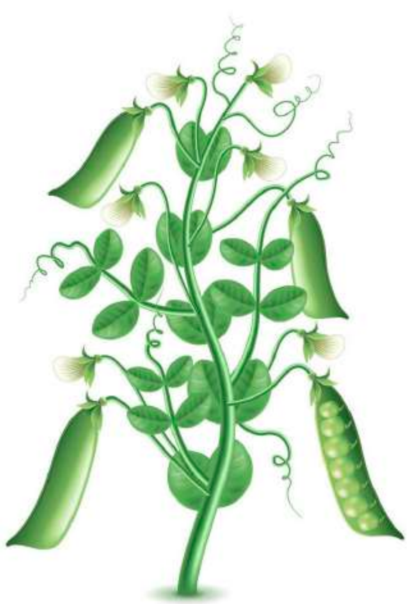
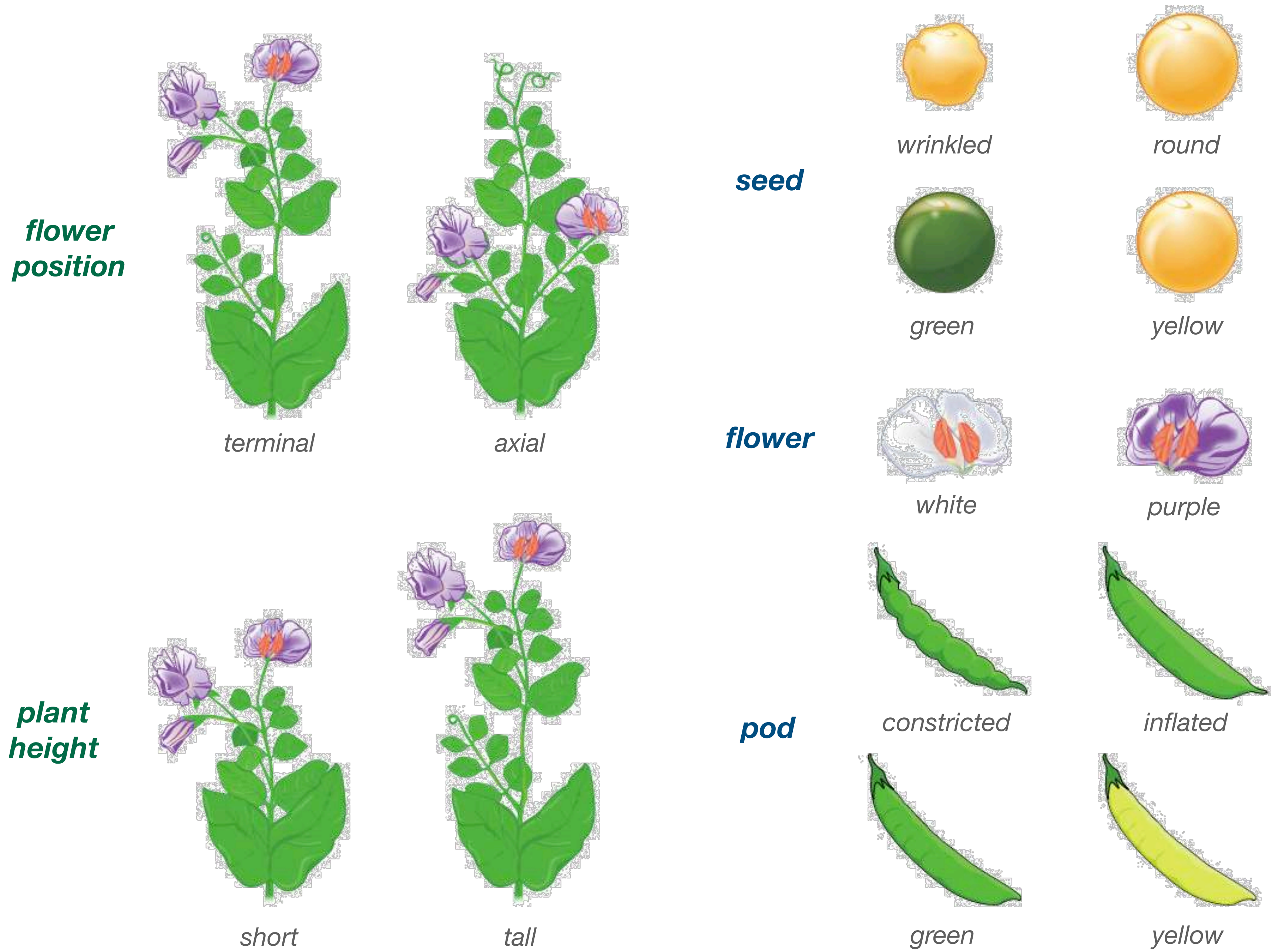


pea plant
pisum sativum



Genetics can be… complicated

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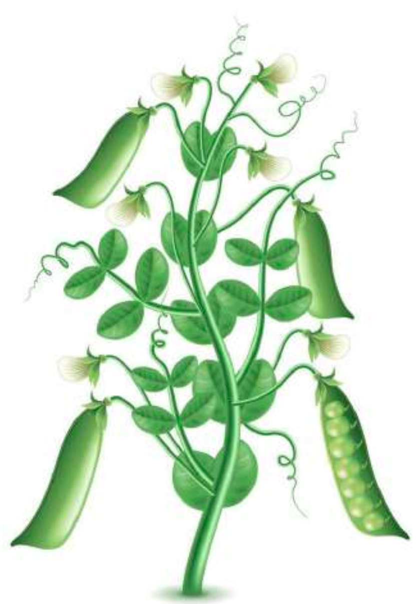
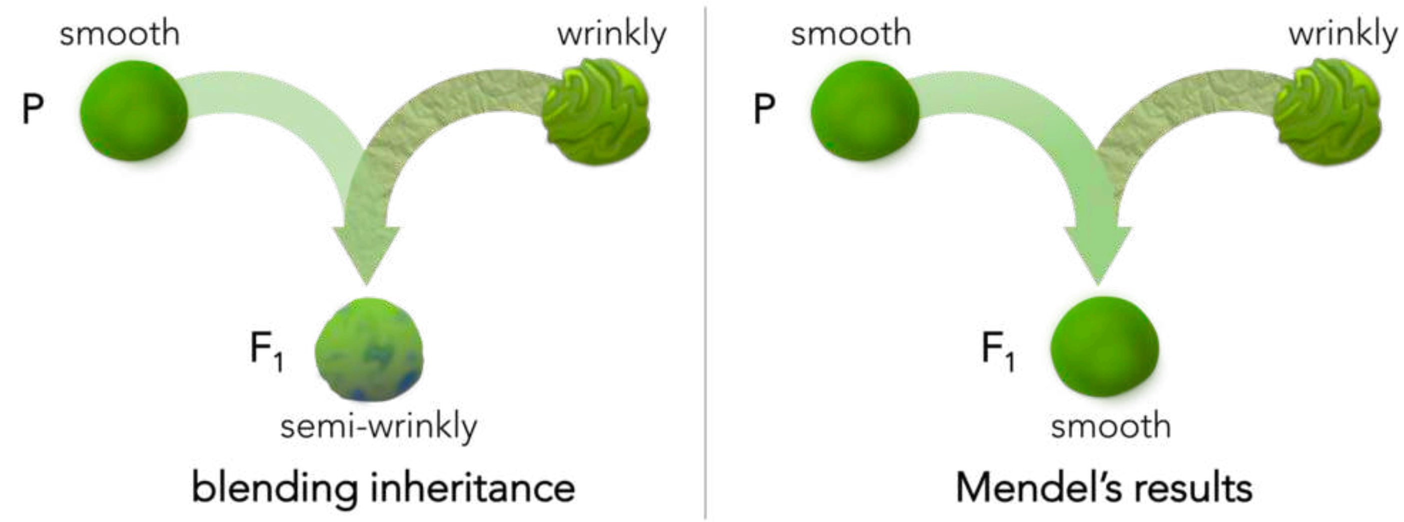
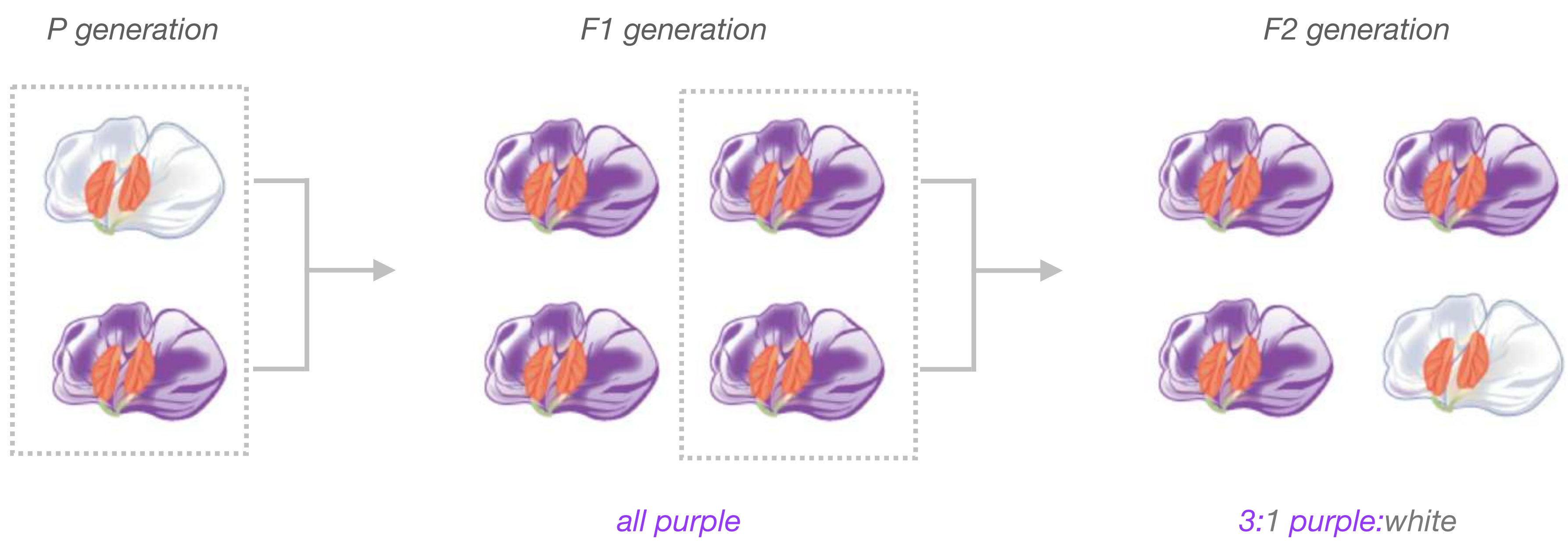


pea plant
pisum sativum





Genetics can be… complicated

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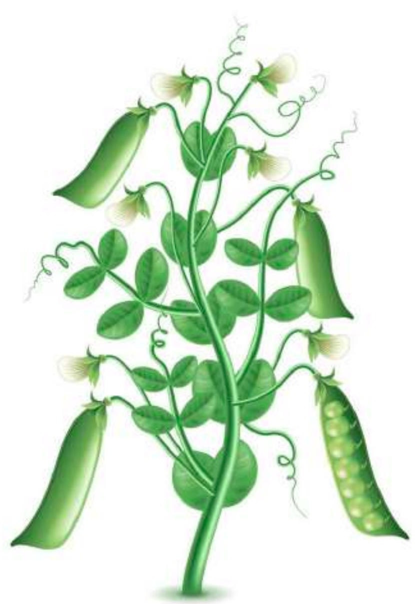
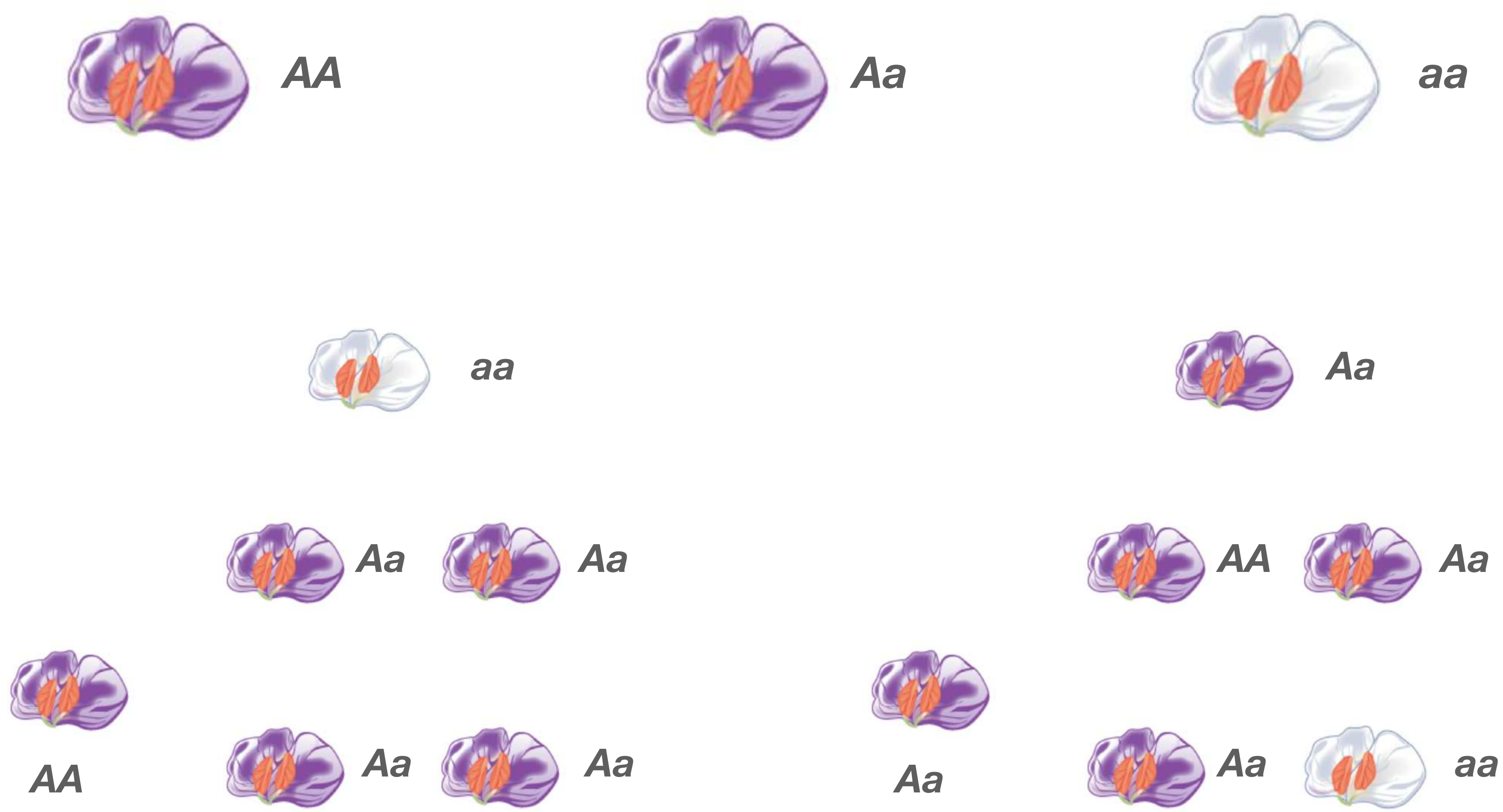


pea plant
pisum sativum

A = dominant allele ***a = recessive allele***



Mendelian inheritance

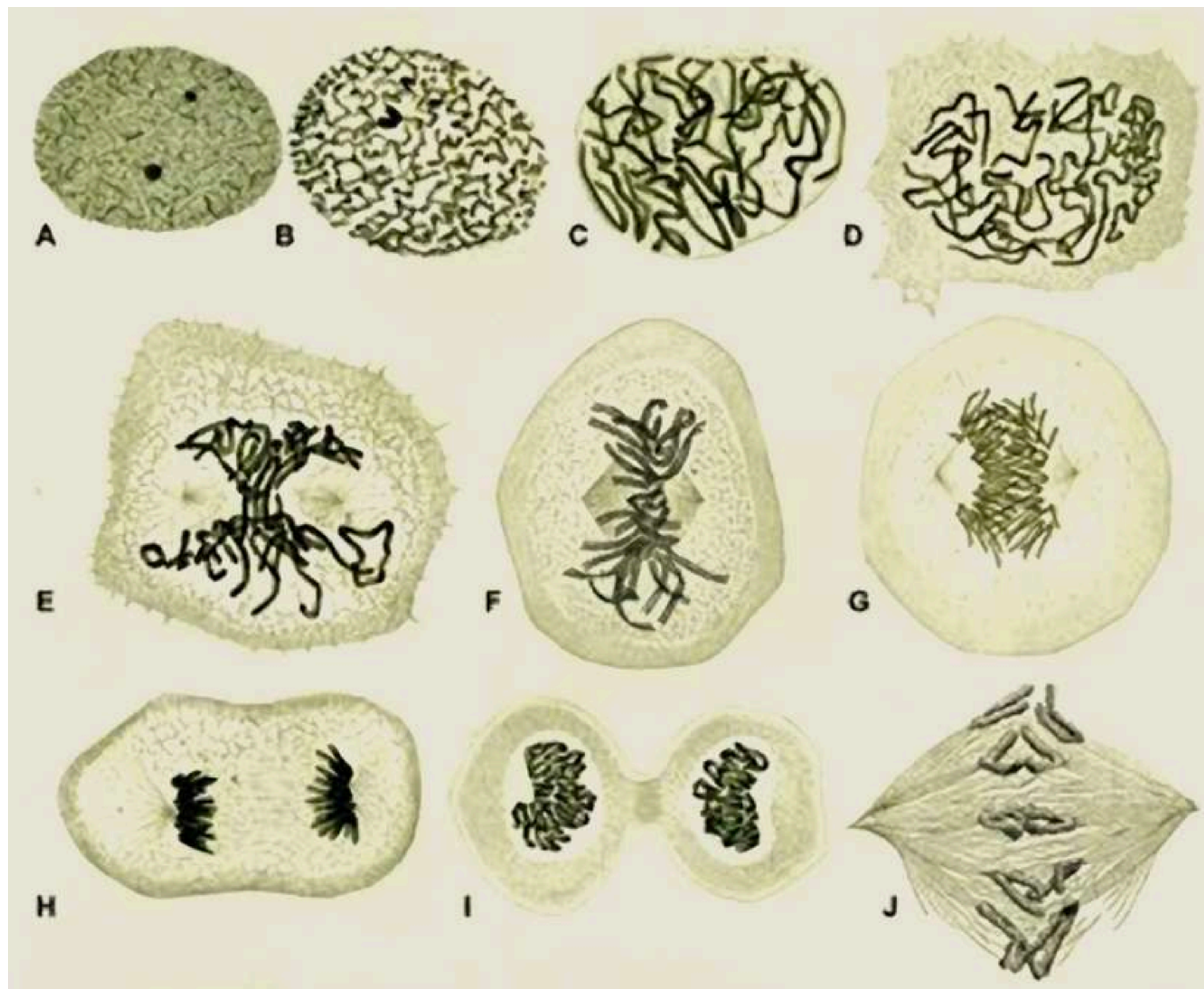
Molecular Genetics

1882

Walter Flemming

"Further study of the division phenomena… reveals a stainable substance of the nucleus. I shall coin the term ***chromatin*** for the time being."

chromosomes as the physical hereditary molecule?



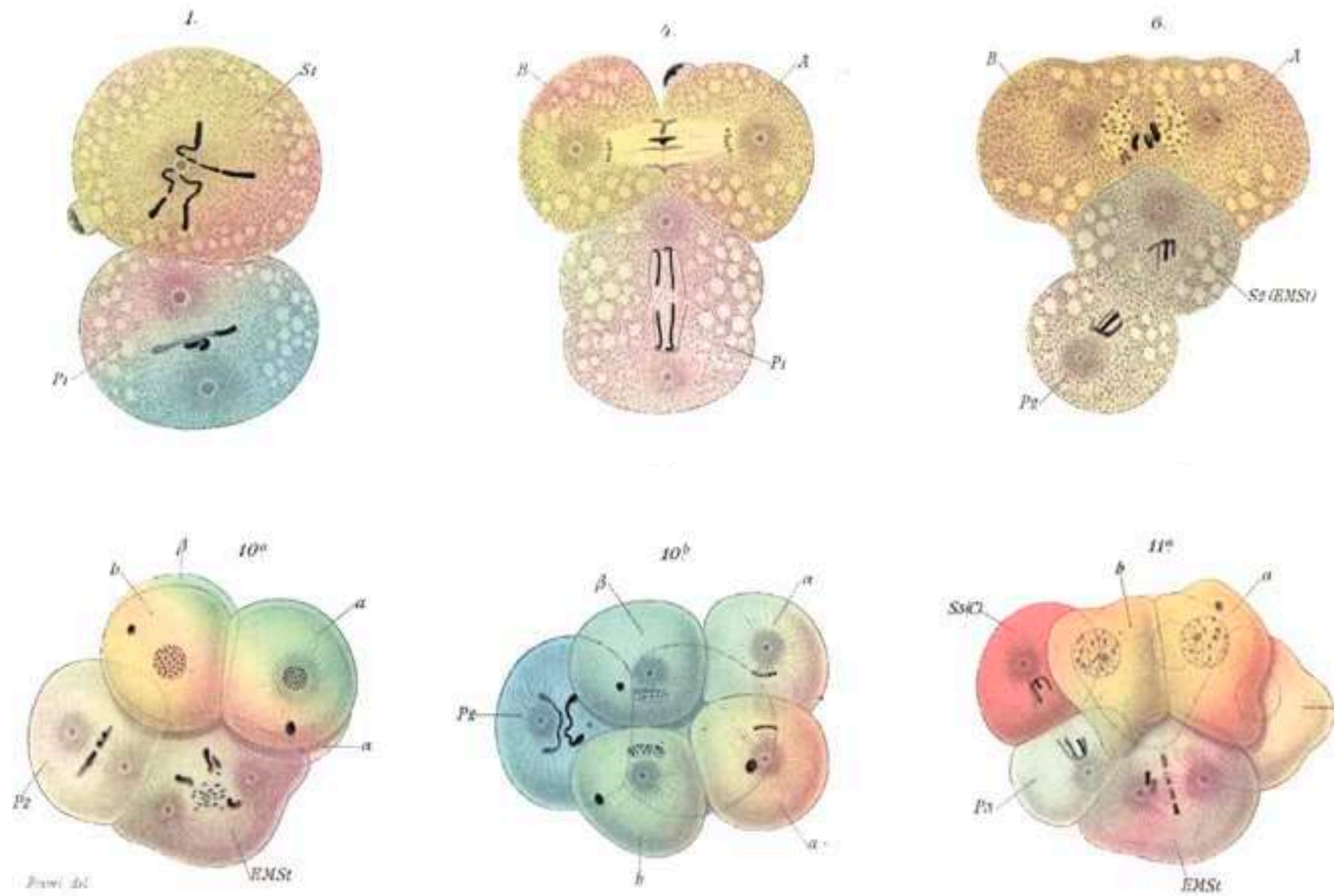
Flemming, Walther. *Zellsubstanz, kern und zelltheilung*. Vogel, 1882.

Molecular Genetics

1888

Theodor Bovori

chromosomes are copied and sometimes ***assymetrically distributed***



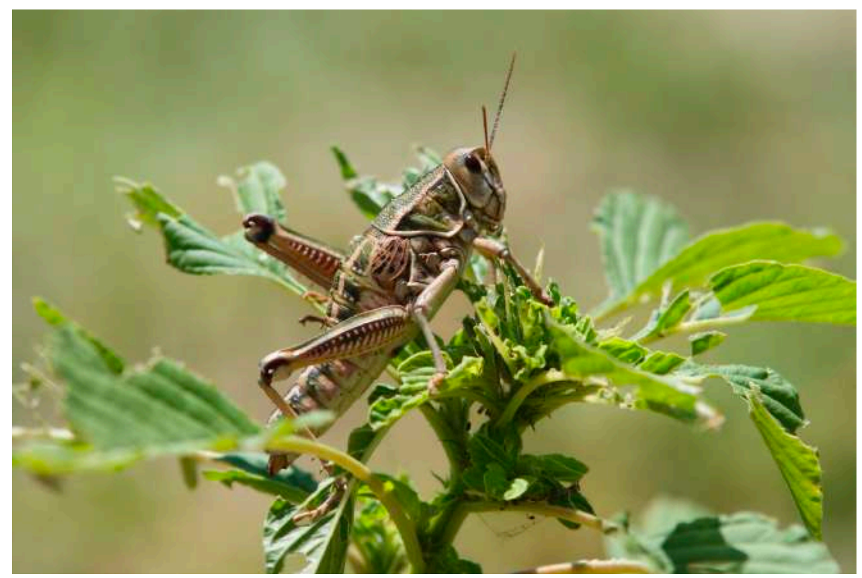
Boveri, T. 1888.

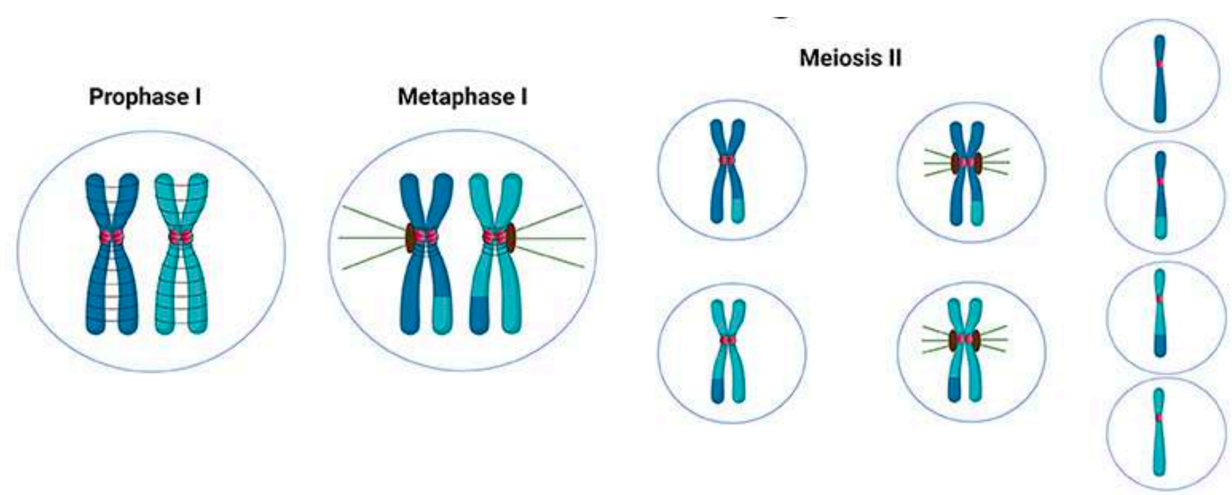
Molecular Genetics

1903

Walter Sutton

“germ cells” contain half of chromosomes - fertilization completes the set





Sutton, Walter S. *The Biological Bulletin,* **1903**, 231-250.

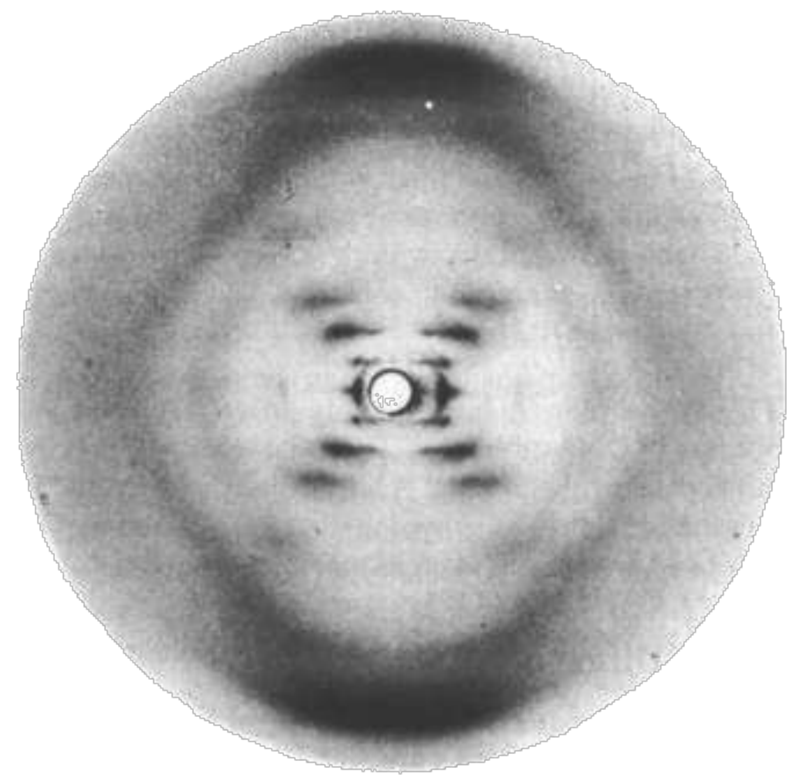
Molecular Genetics

1951

chemical structure of DNA - how is information encoded?

Rosalind Franklin

Maurice Wilkins



- *must be helical in shape*
- *water is excluded from interior*
- *ratio of A = T, C = G*

Nature, **1953**, 171, 740-741.

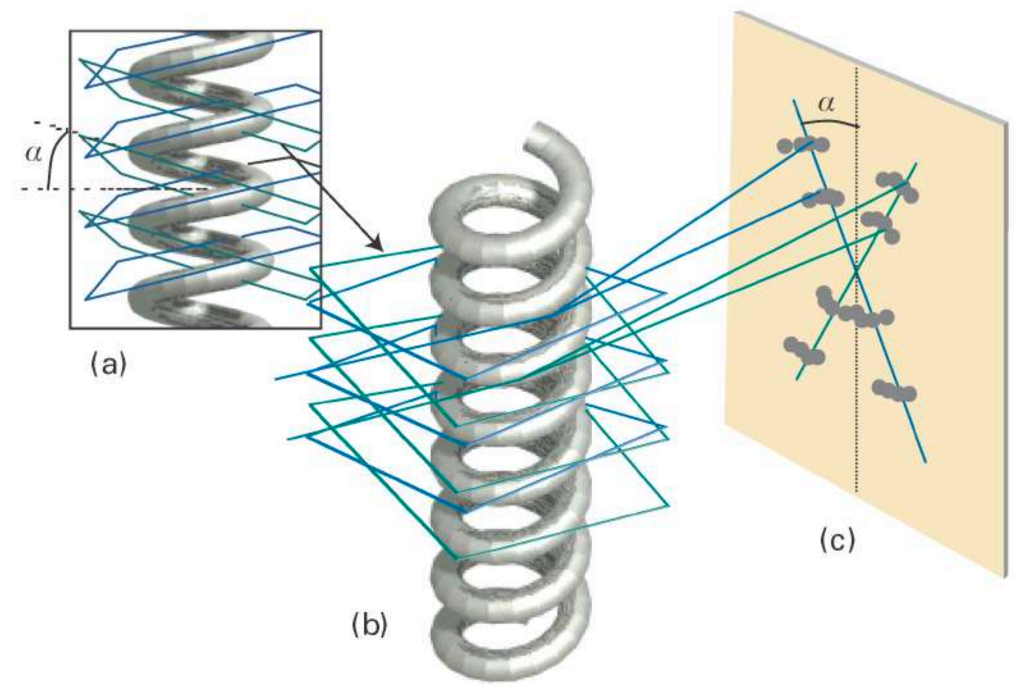
Molecular Genetics

1951

Rosalind Franklin

Maurice Wilkins

chemical structure of DNA - how is information encoded?



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Nature, **1953**, 171, 740-741.

Molecular Genetics

1951

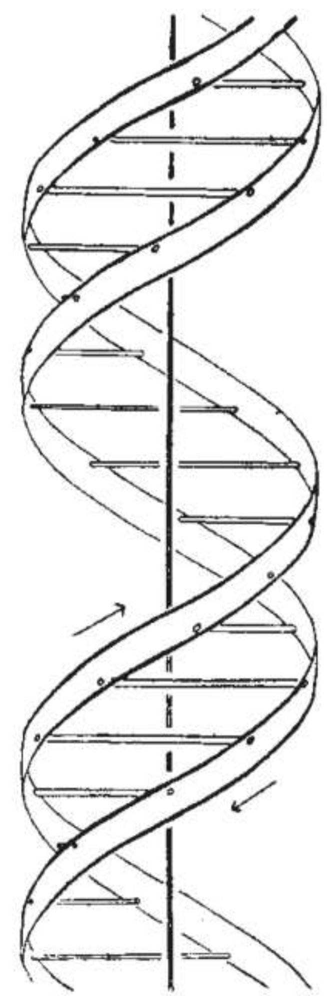
chemical structure of DNA - how is information encoded?

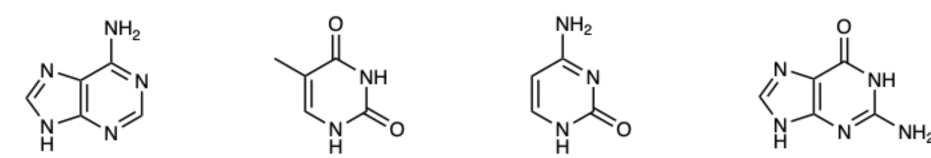
Rosalind Franklin

Maurice Wilkins

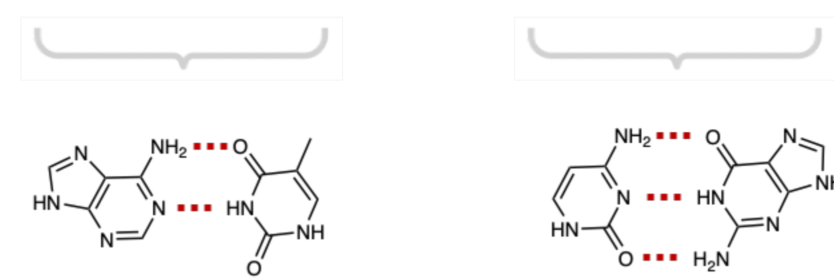
James Watson

Francis Crick





adenosine (A) thymine (T) cytidine (C) guanine (G)



A : T

C : G

Nature, **1953**, 171, 737-738.

Molecular Genetics

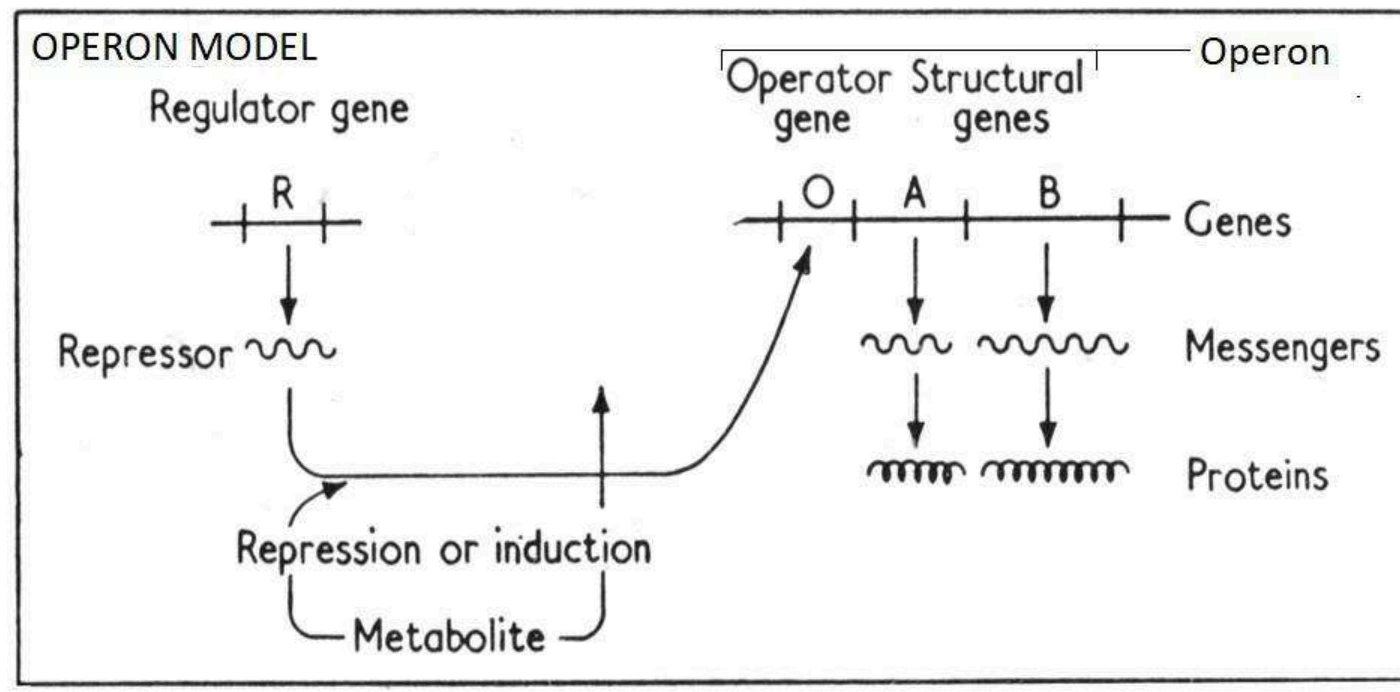
1961

messenger RNA is made from DNA, enables protein synthesis?

Sydney Brenner

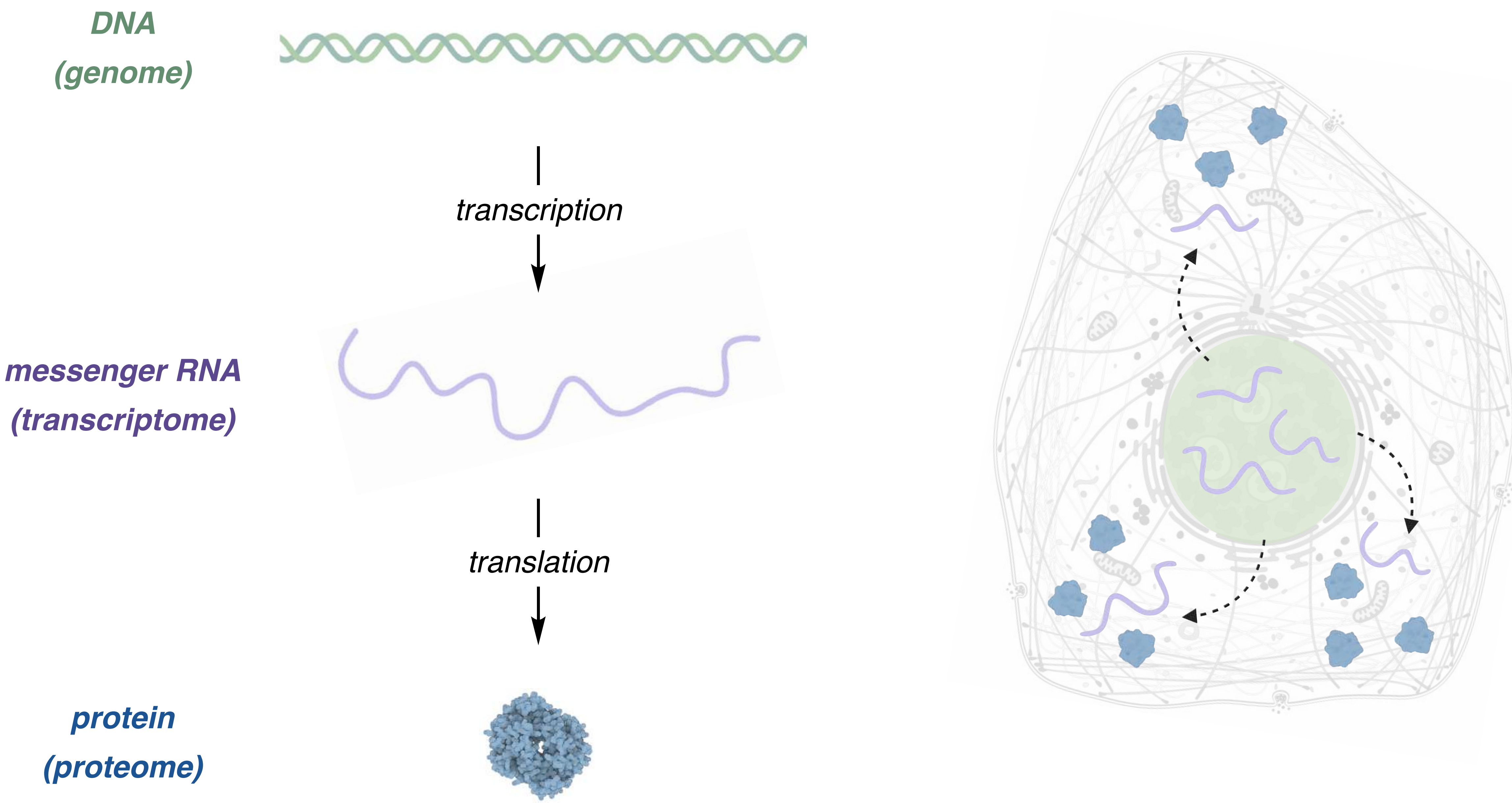
Francis Crick

Francois Jacob



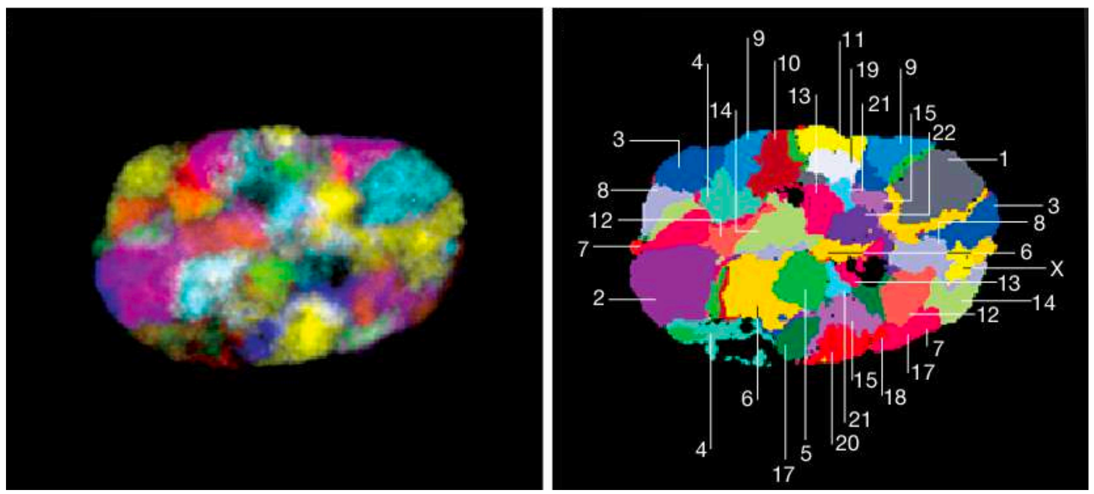
Nature, **1961,**190, 581-585.

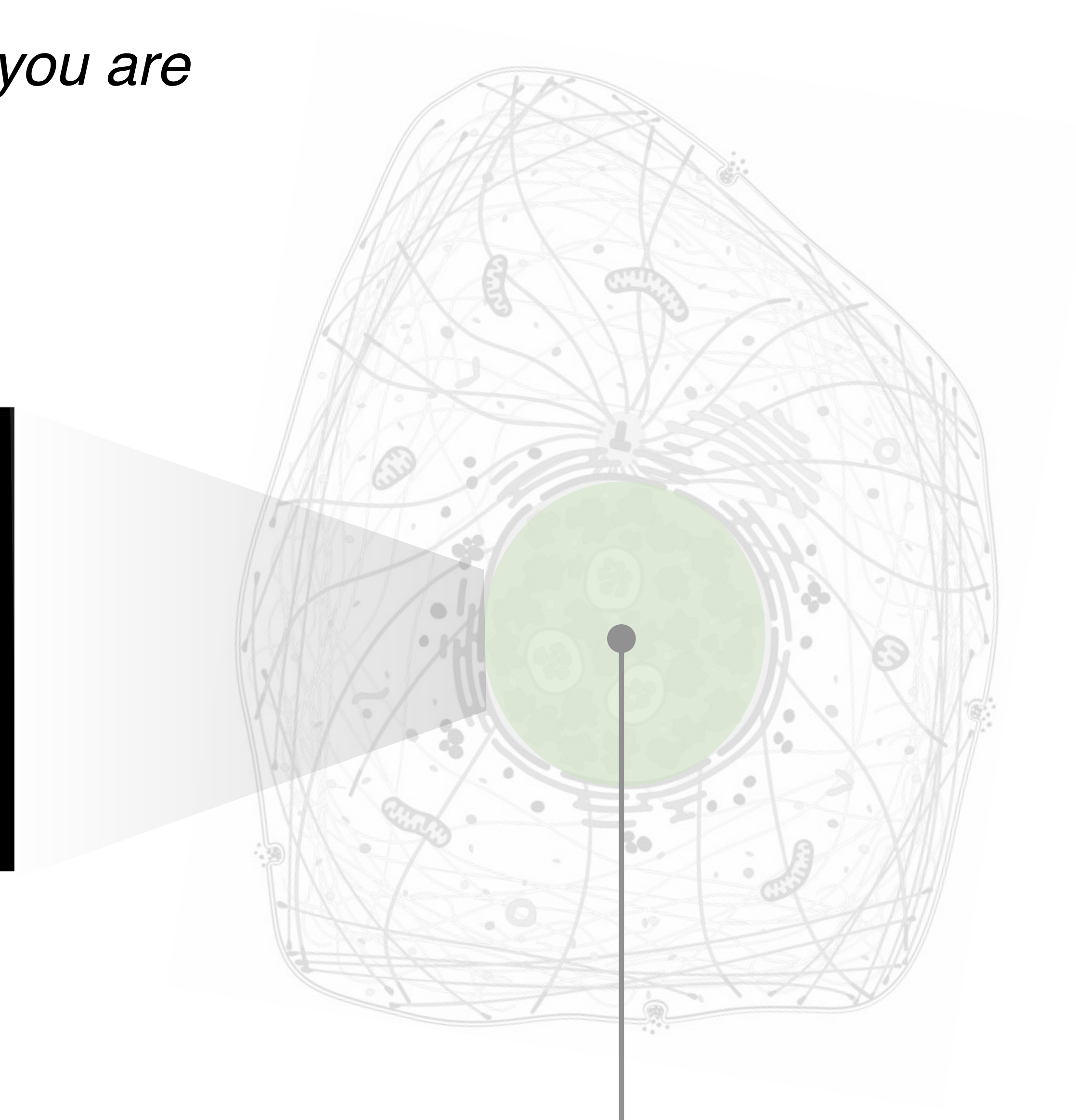
The "you" you are

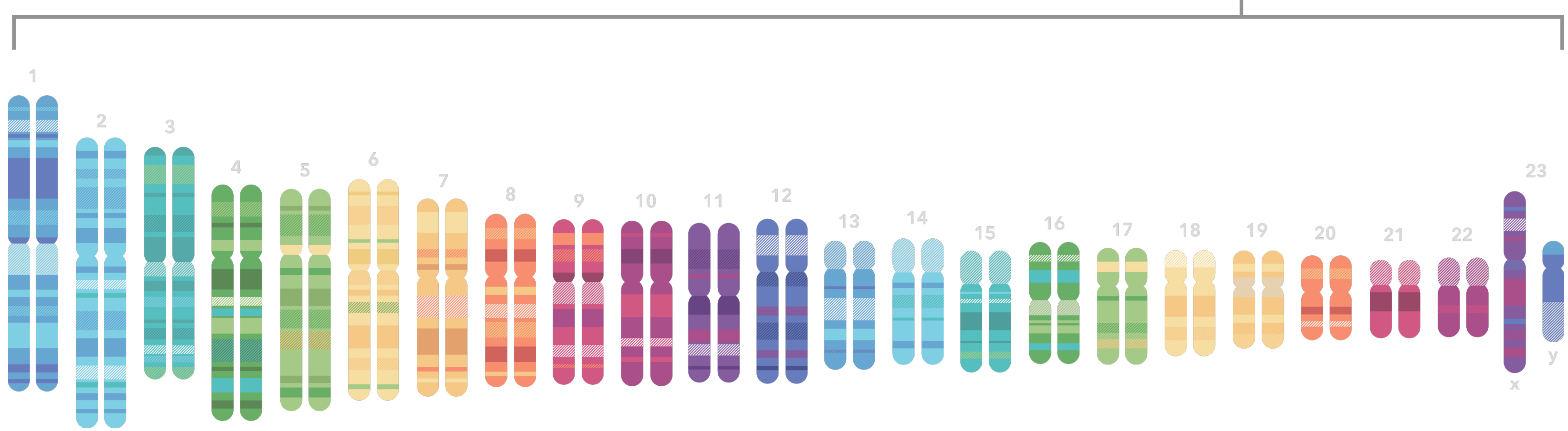


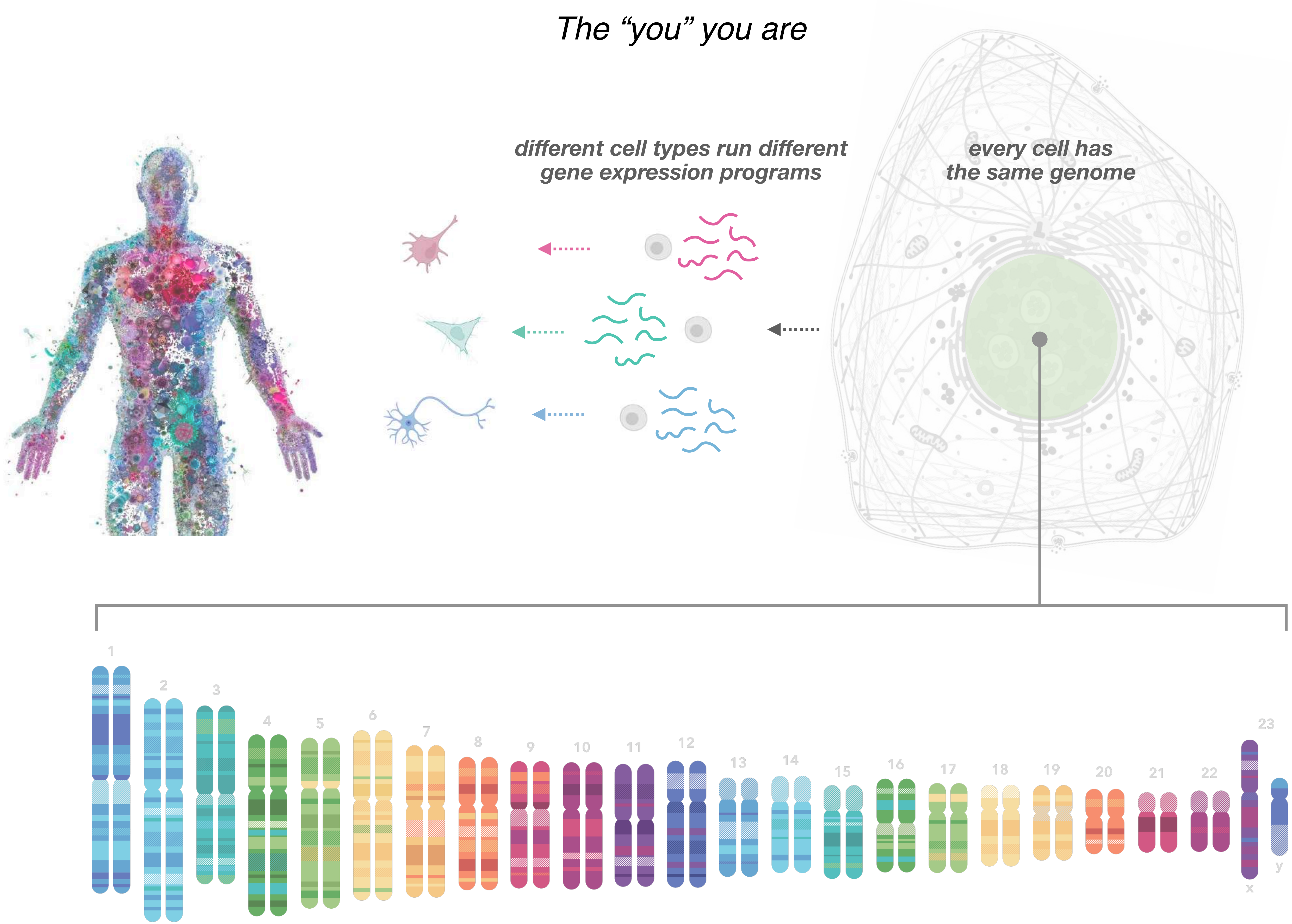
The “you” you are

chromosomes occupy discrete terrorites in the nucleus



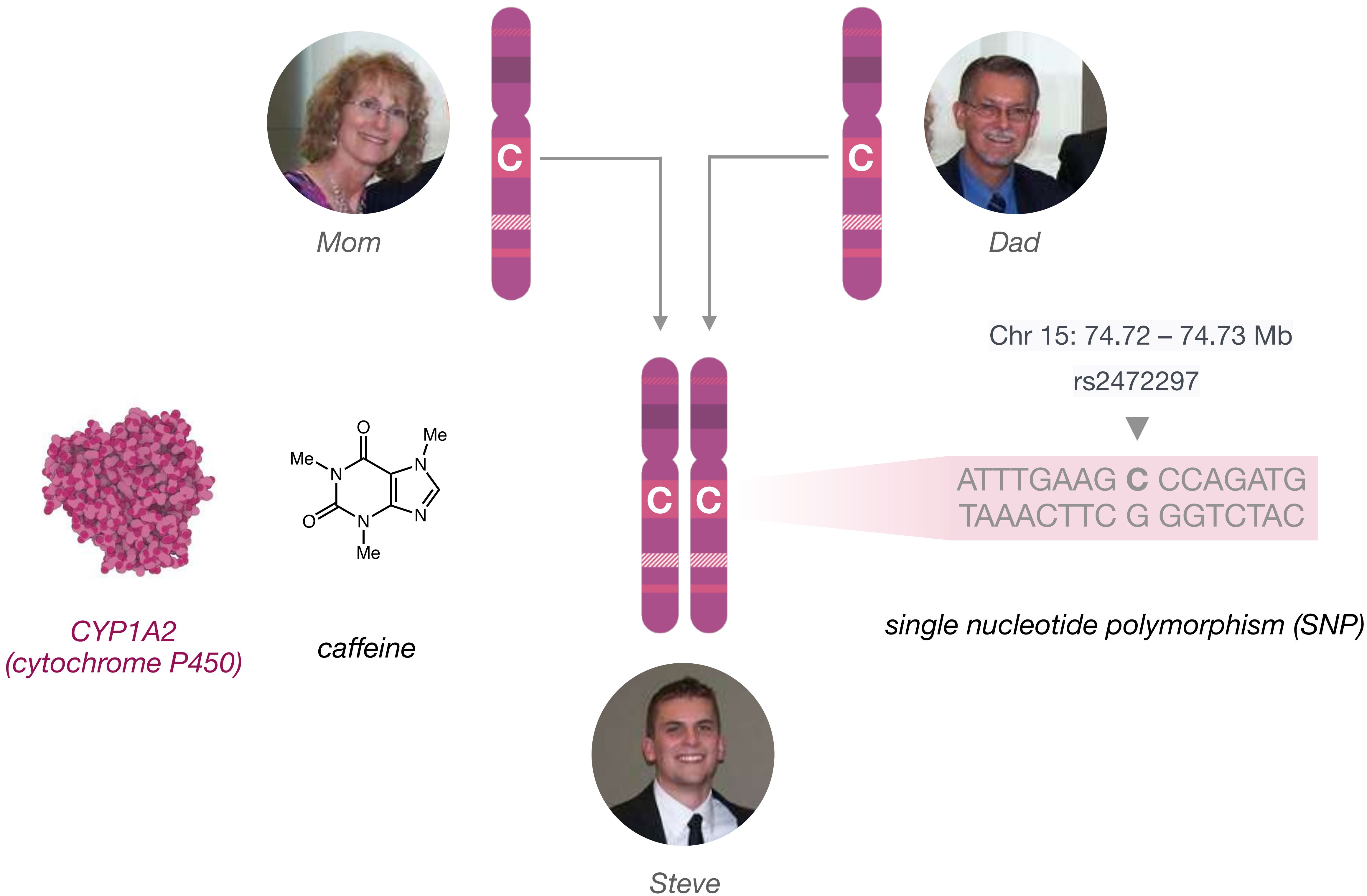




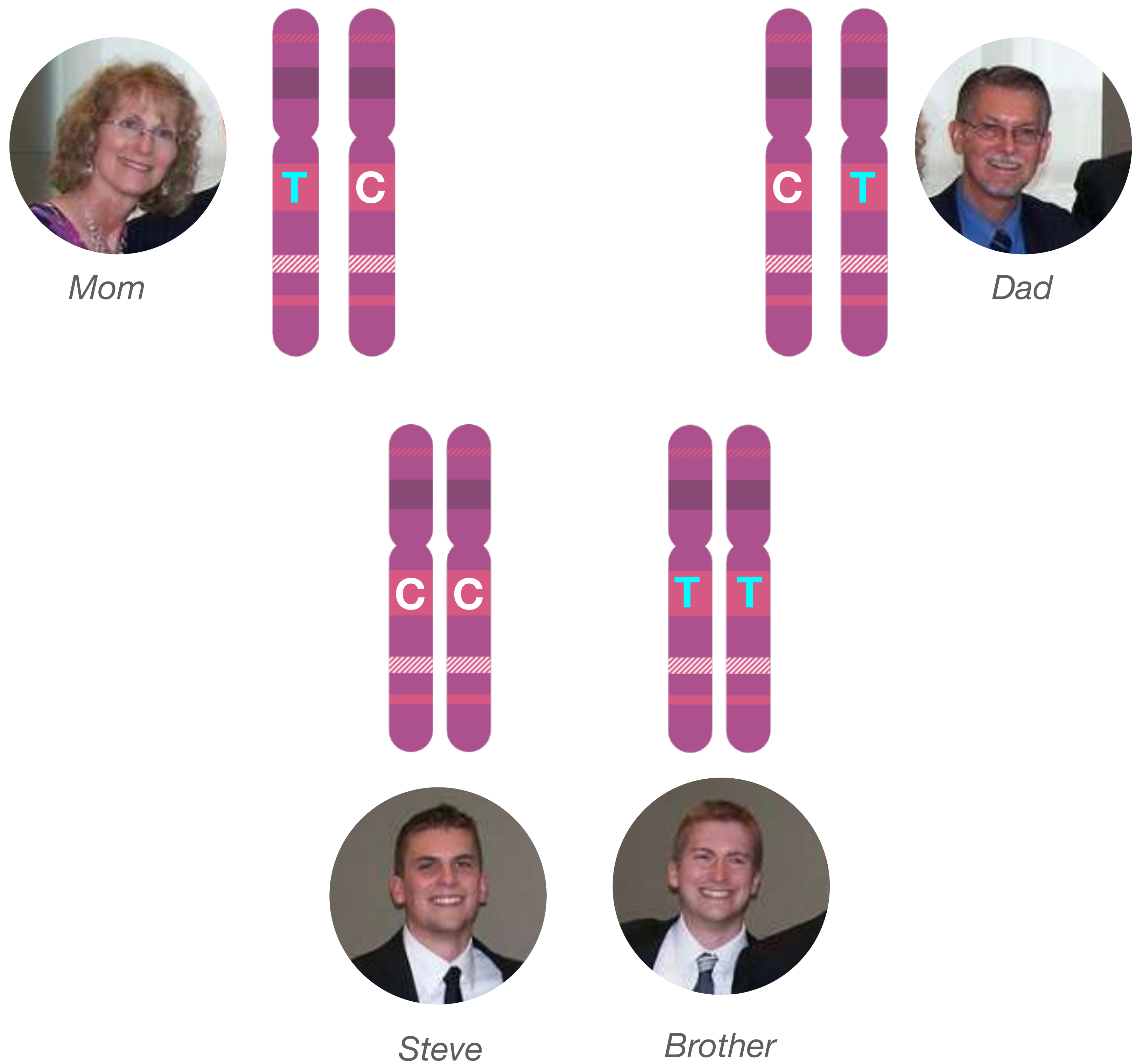


The "you" you are
different cell types run different gene expression programs
every cell has the same genome
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
x
y

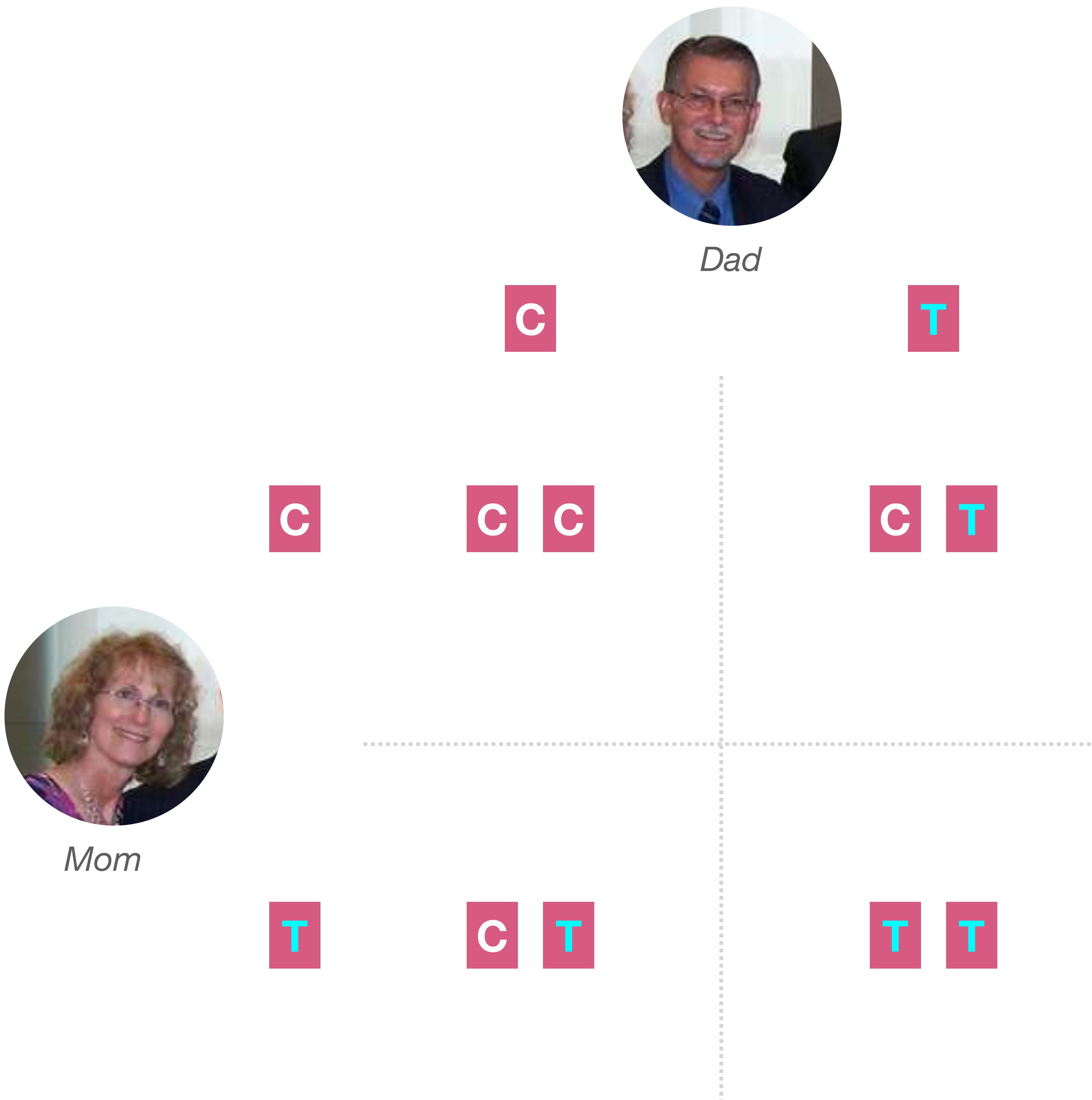
The "you" you are



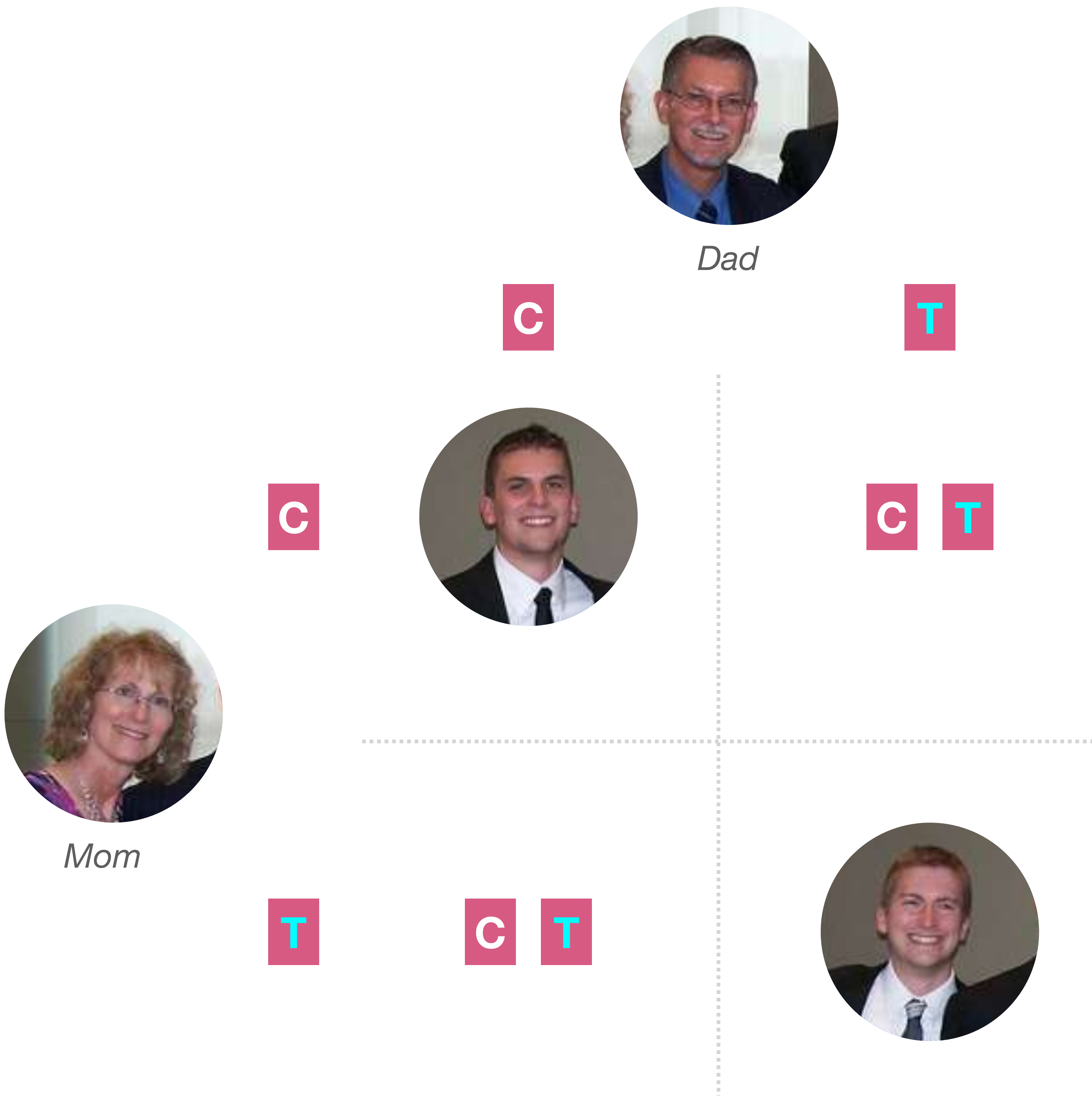
The "you" you are



The “you” you are



The “you” you are



The "you" you are

Blood Type

	A	B	AB	O
Red Blood Cell Type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red blood Cell	A antigen	B antigen	A and B antigens	None
Blood Types Compatible in an Emergency	A, O	B, O	A, B, AB, O (AB^+ is the universal recipient)	O (O is the universal donor)

The “you” you are

Dad

Mom

	B	O
A	A B	A O
O	B O	O O

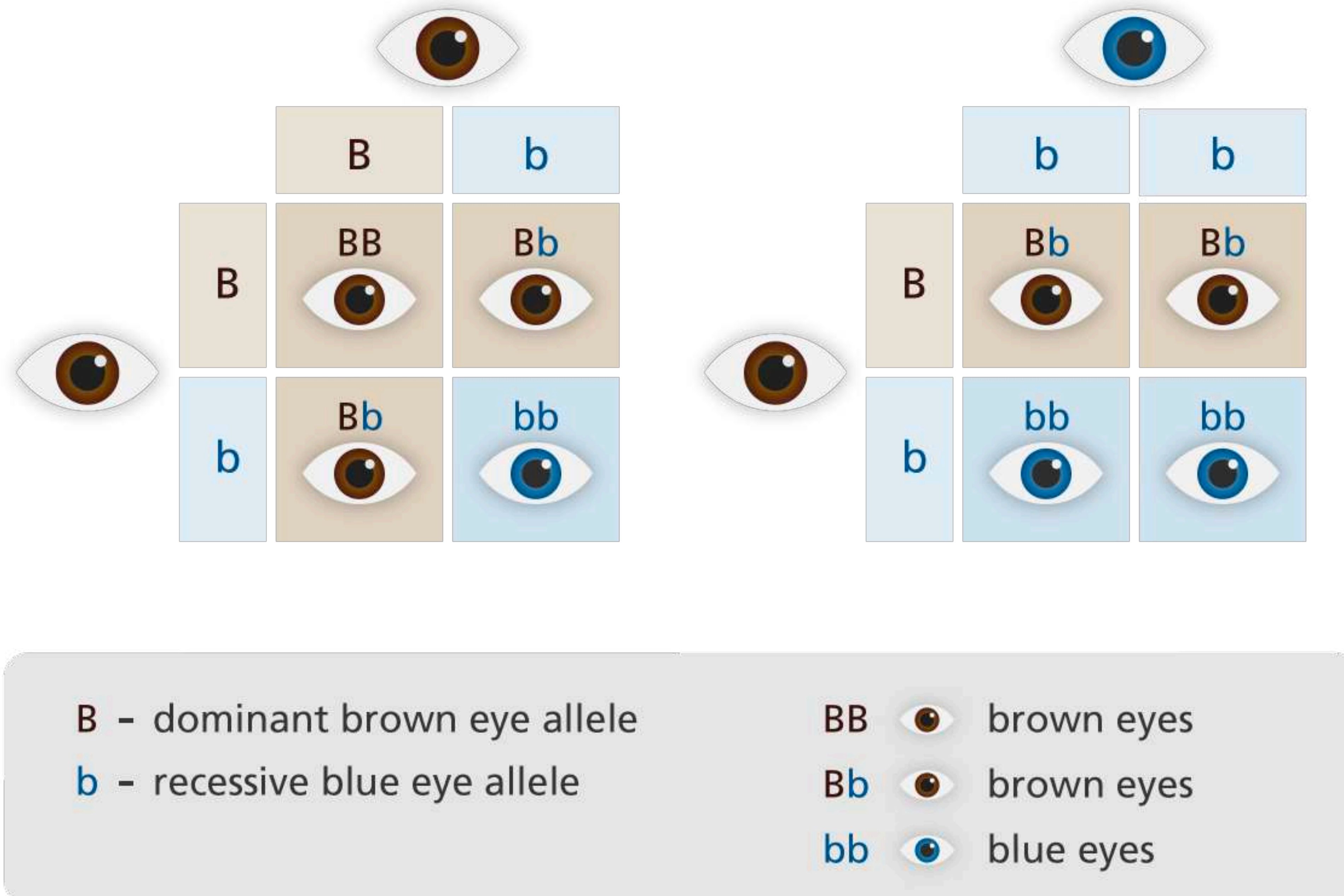
The “you” you are

Dad

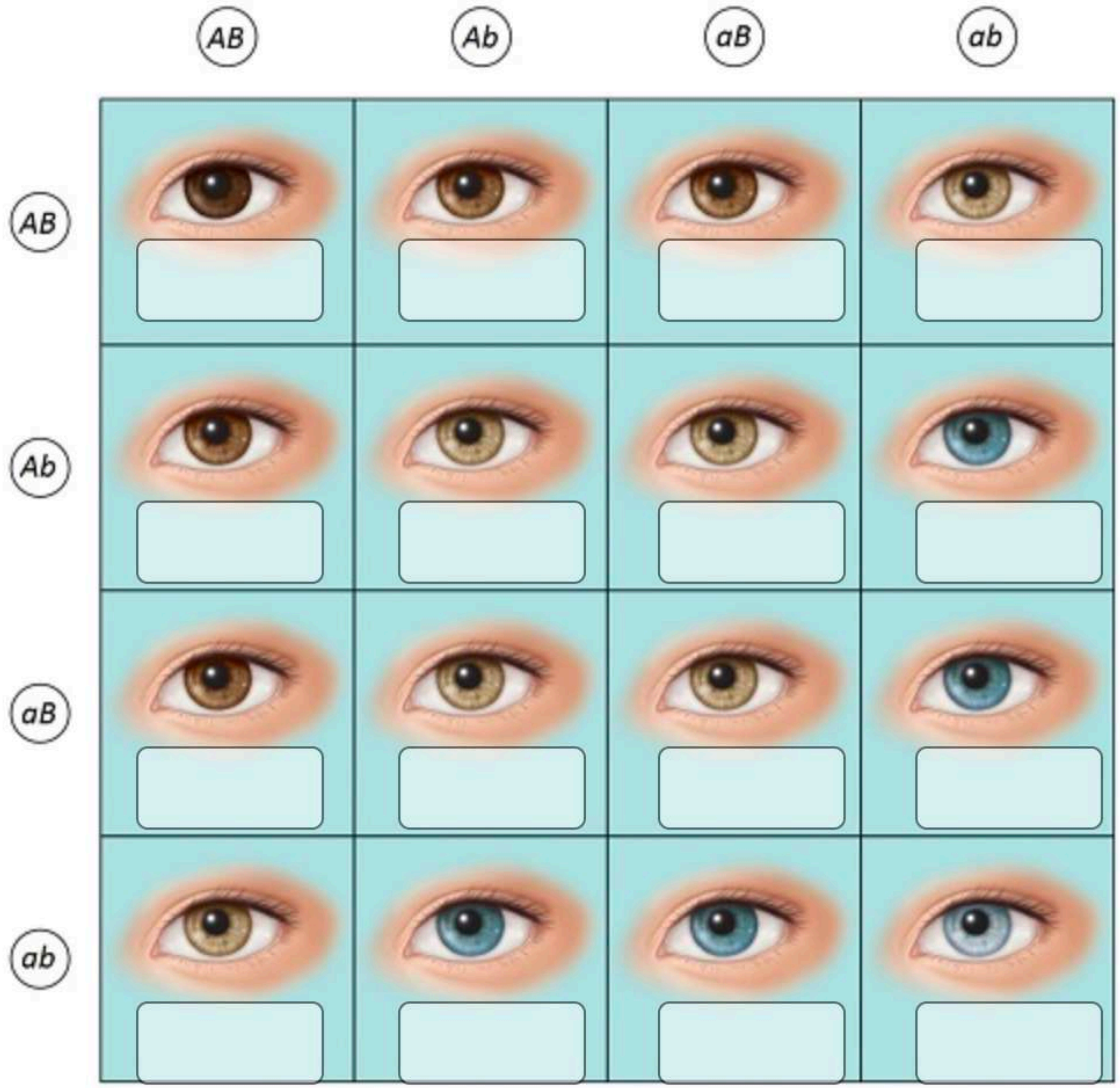
	B	O
A	A B	A O
O	B O	O O

Mom

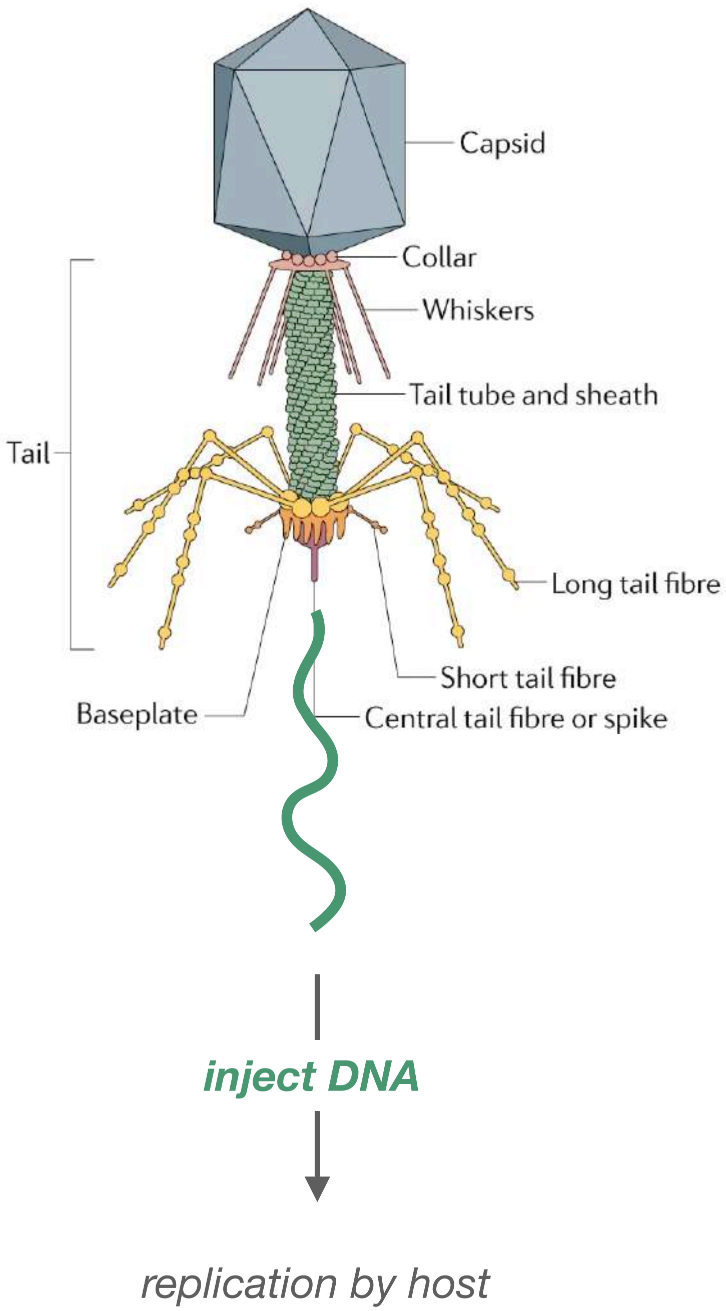
The "you" you are



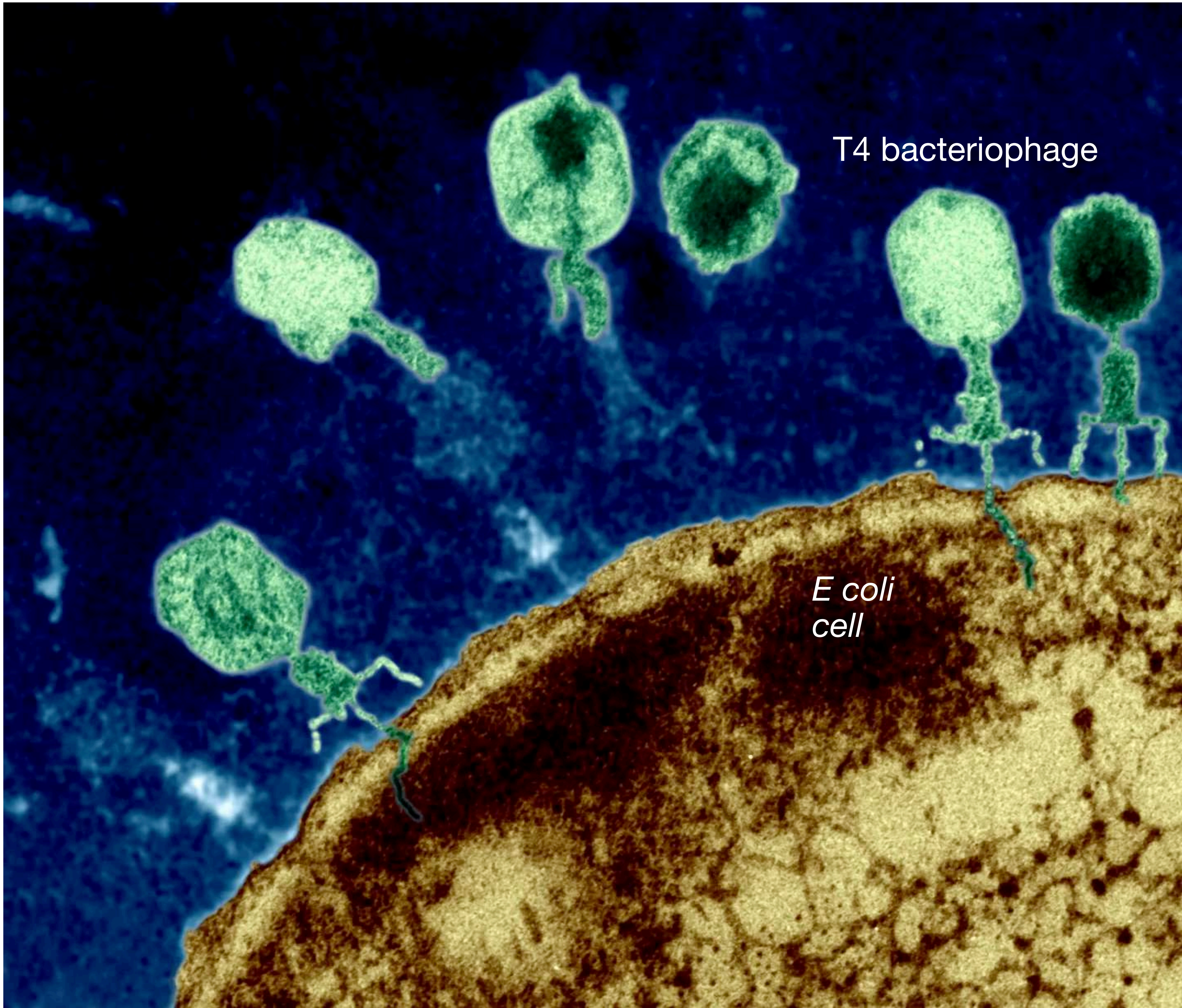
The "you" you are



The microbial arms race



Nat. Rev. Microbiol., **2015,** 13(*12*) 777-786.

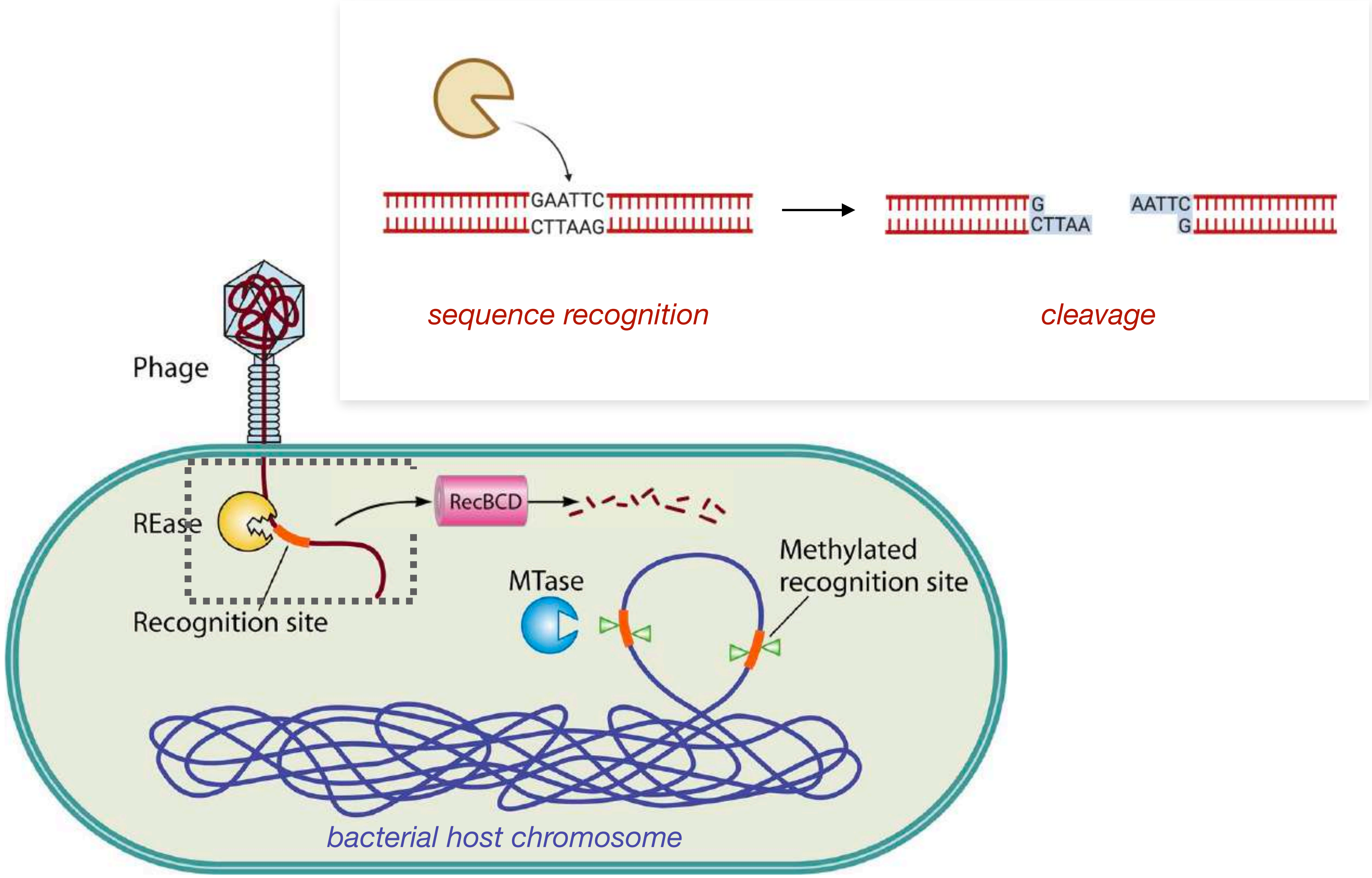


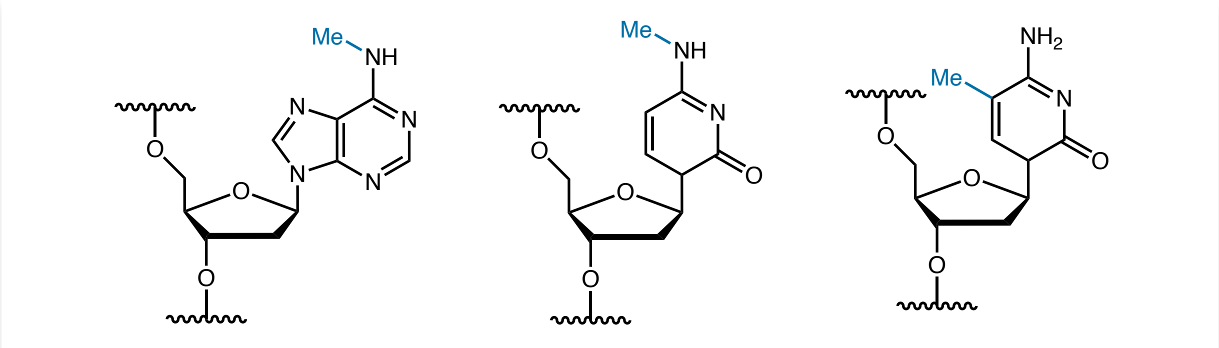
Restriction enzymes

Werner Arber

Daniel Nathans

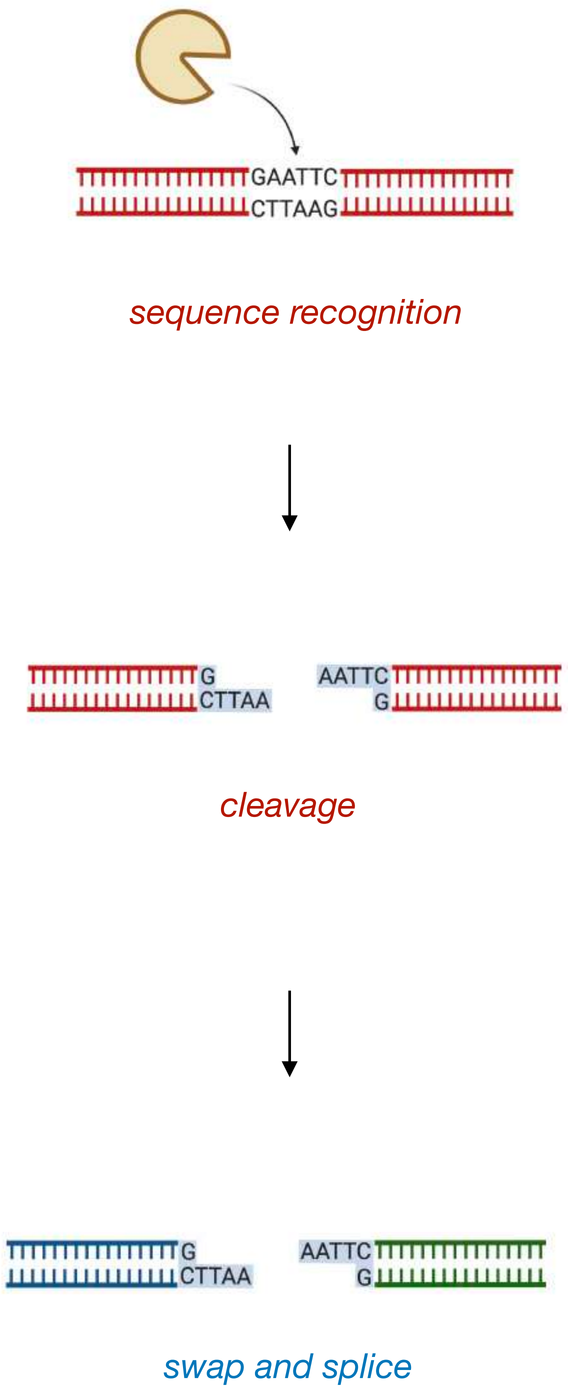
Hamilton Smith

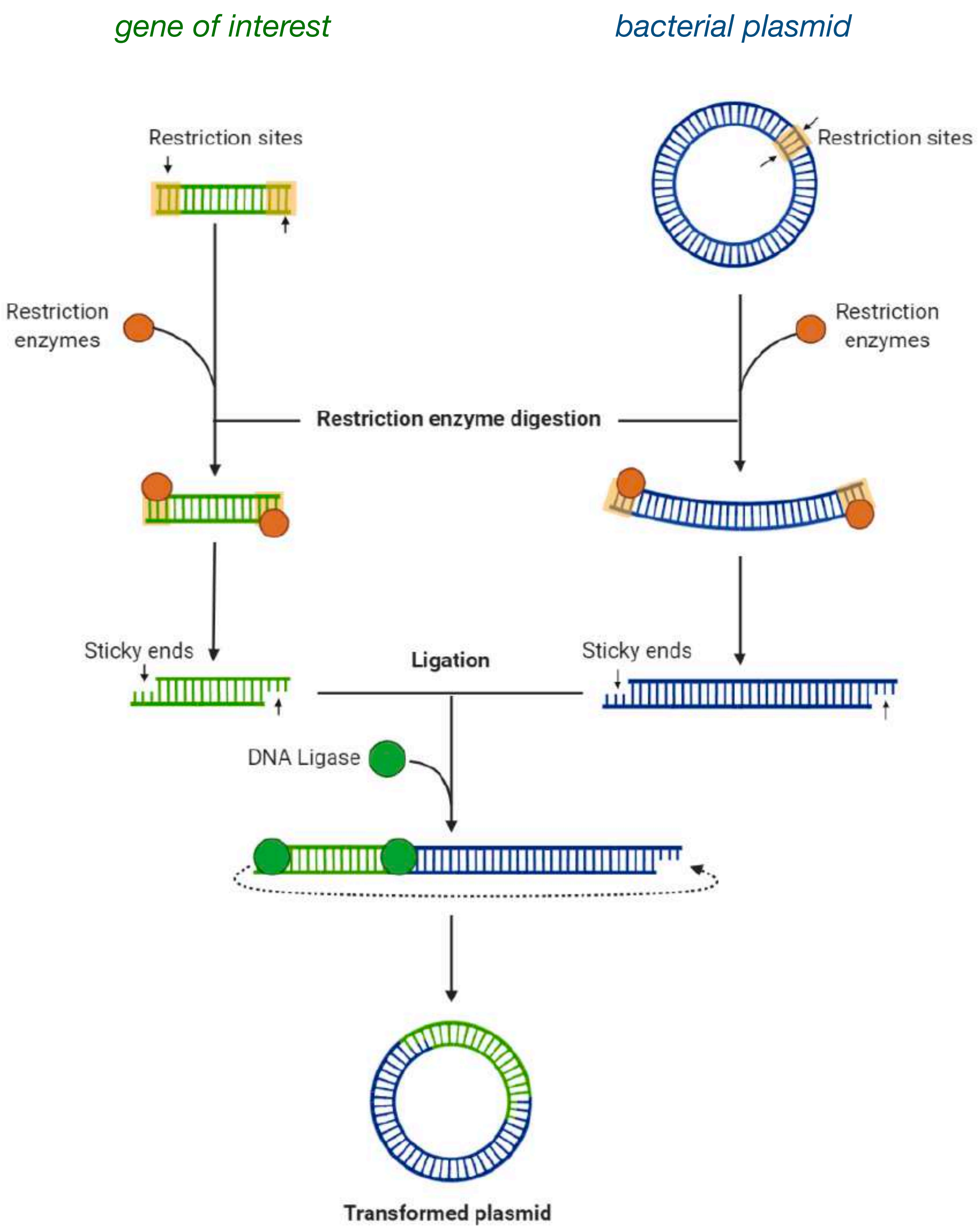




Nat. Rev. Microbiol., **2015**, 13(*12*) 777-786.

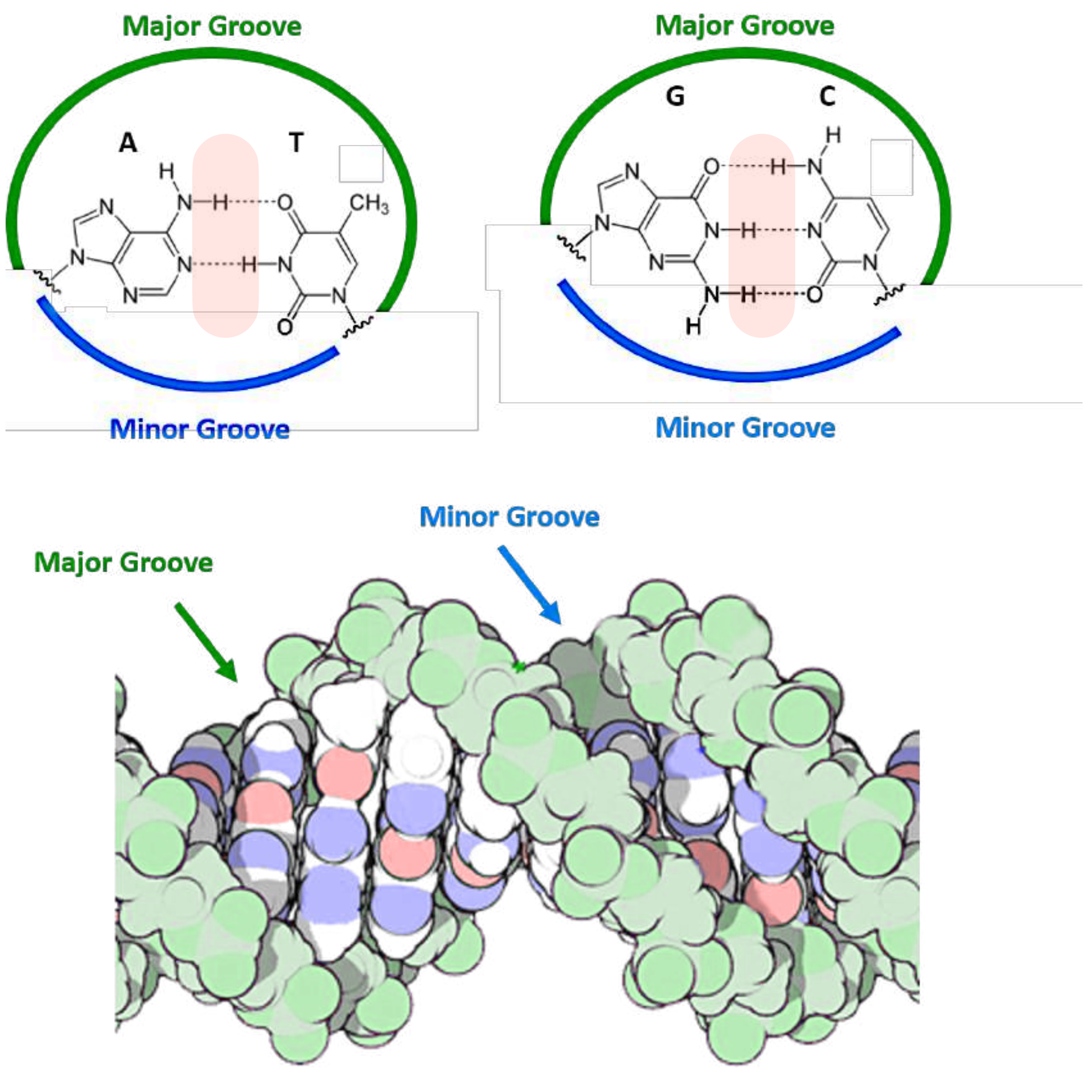
Genetic engineering with restriction enzymes

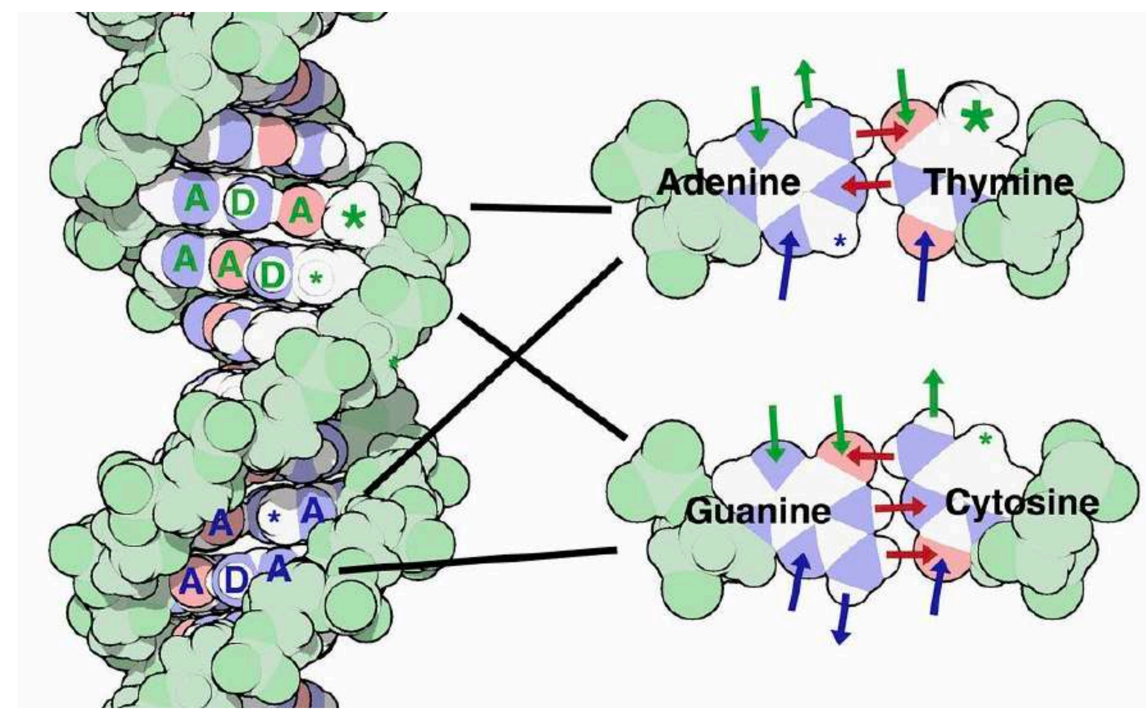




Problem 1: restriction enzymes are permanently locked in on their targets

5´. . . G▼G A T C C . . . 3´
3´. . . C C T A G▲G . . . 5´





programming recognition of new sites requires complete redesign/evolution of enzyme

Problem 2: the recognition sequences are not that unique

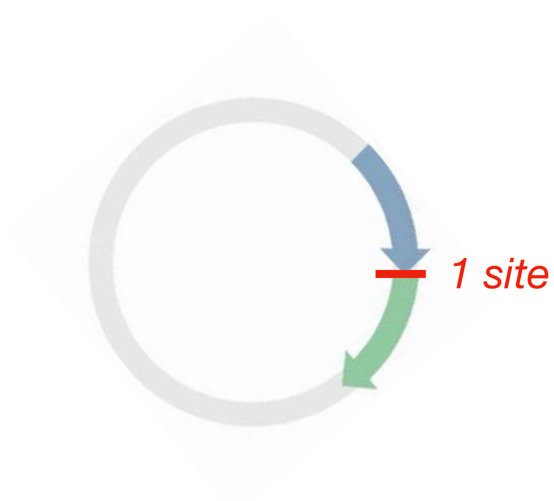
5´... G▼GATCC...3´
3´... CCTAG▲G...5´

bacterial plasmid

~6,000 nt

(1/4)⁶ = 0.02% probability

0.02% x 6000 = ***1.2 sites***

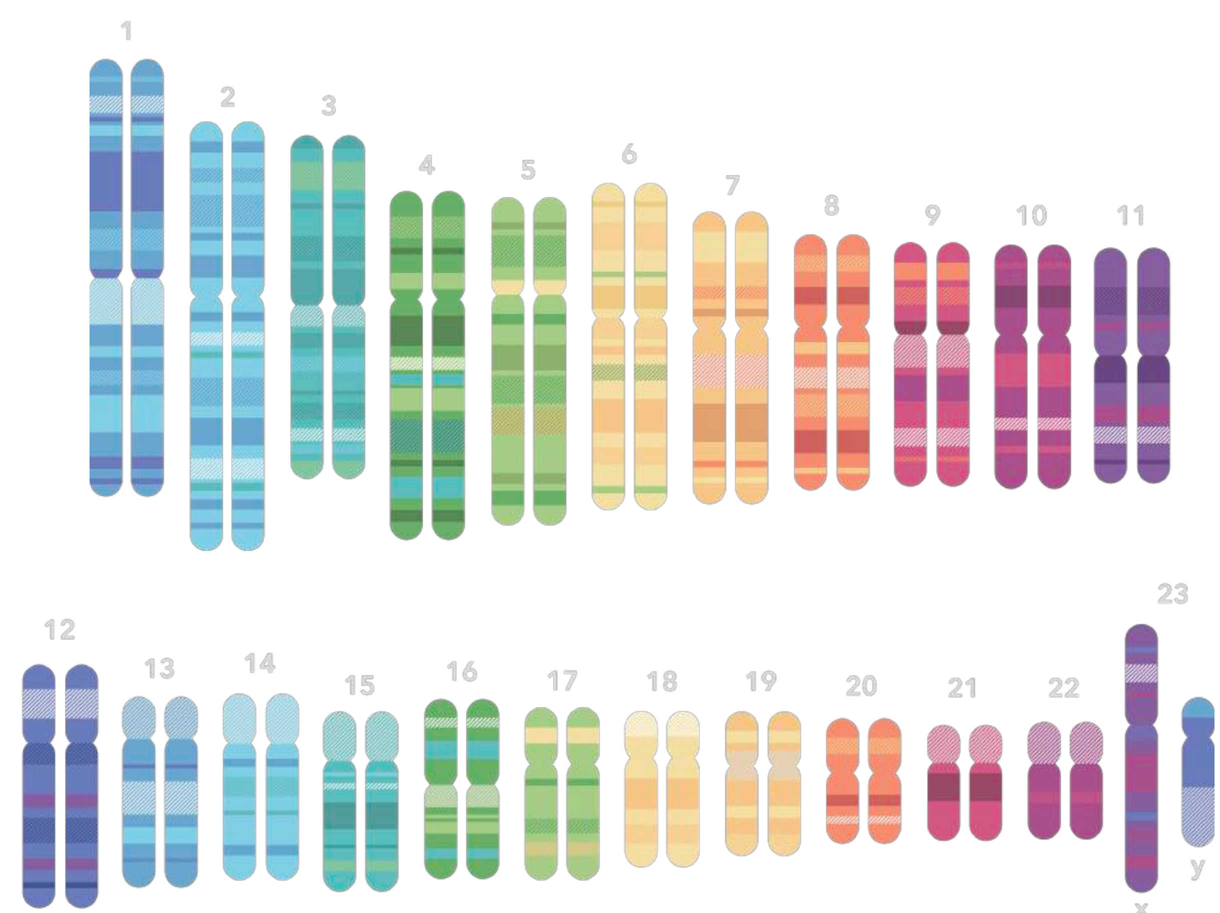


human genome

~6,000,000,000 nt

(1/4)⁶ = 0.02% probability

0.02% x 6,000,000,000 = ***1,200,000 sites***



Meanwhile, in Japan

Yoshizumi Ishino

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

```
TGAAAATGGGAGGGAGTTCTACCGCAGAGGCGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC (1,451)
   (1,452) CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC (1,512)
   (1,513) CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG (1,573)
   (1,574) CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC (1,634)
   (1,635) CGGTTTATCCCCGCTGGCGCGGGGAACTCG (1,664)

                               GG
 consensus: CGGTTTATCCCCGCT  CGCGGGGAACTC
                               AA
```

"An unusual structure was found in the 3' end flanking region of iap (Fig.5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing, and have been found in *E.coli* and *Salmonella typhimurium* and *mayacttostabilizem*….

…. the biological significance of these sequences is not known."

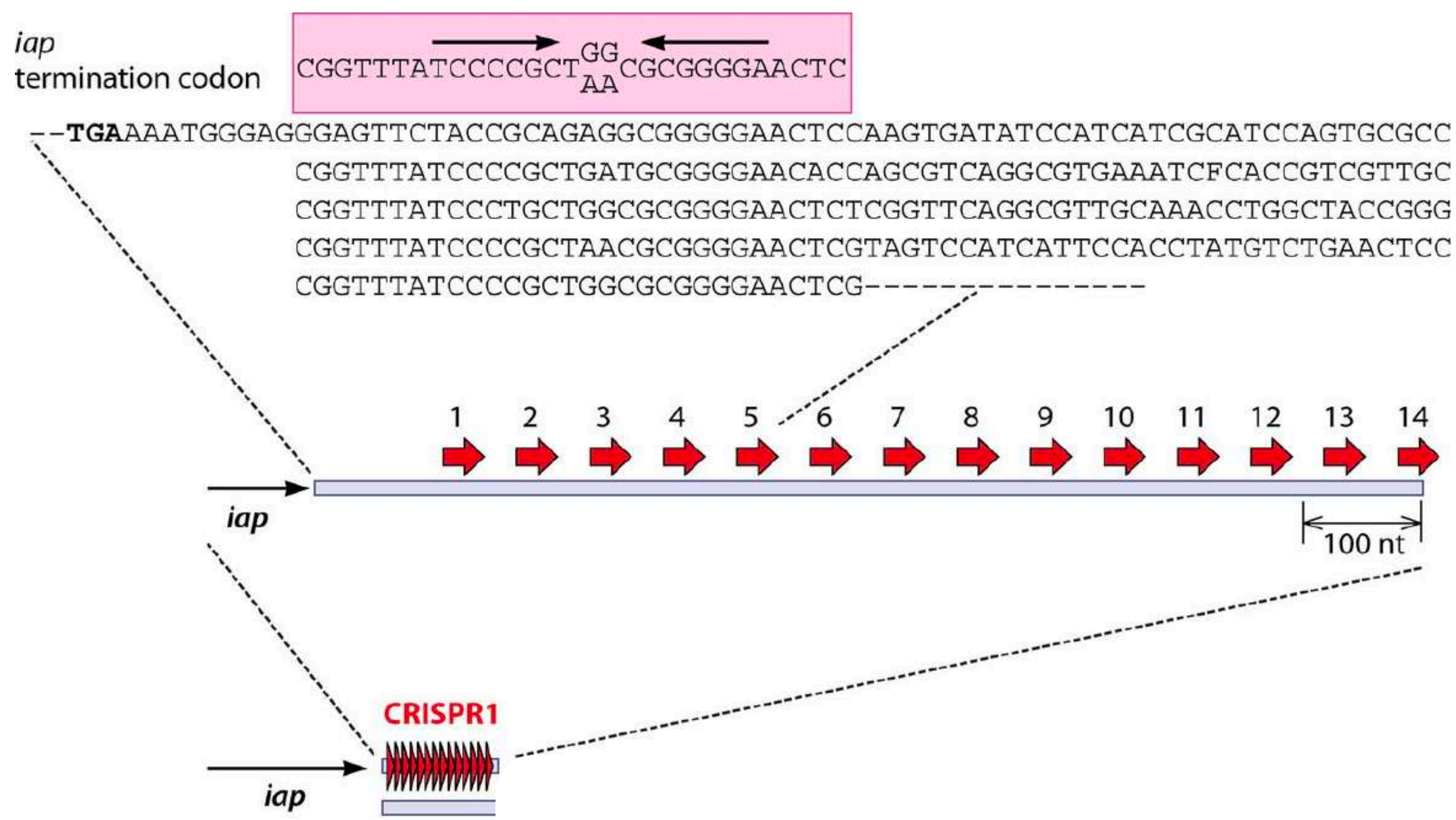
J. Bacteriol., ***1987***, 169(*12*) 5429-5433.

Repetitive sequences

Ruud Jansen

Clustered
Random
Interspersed
Short
Palindromic
Repeats

first 20-30 nt identical *next 30-40 nt always different*



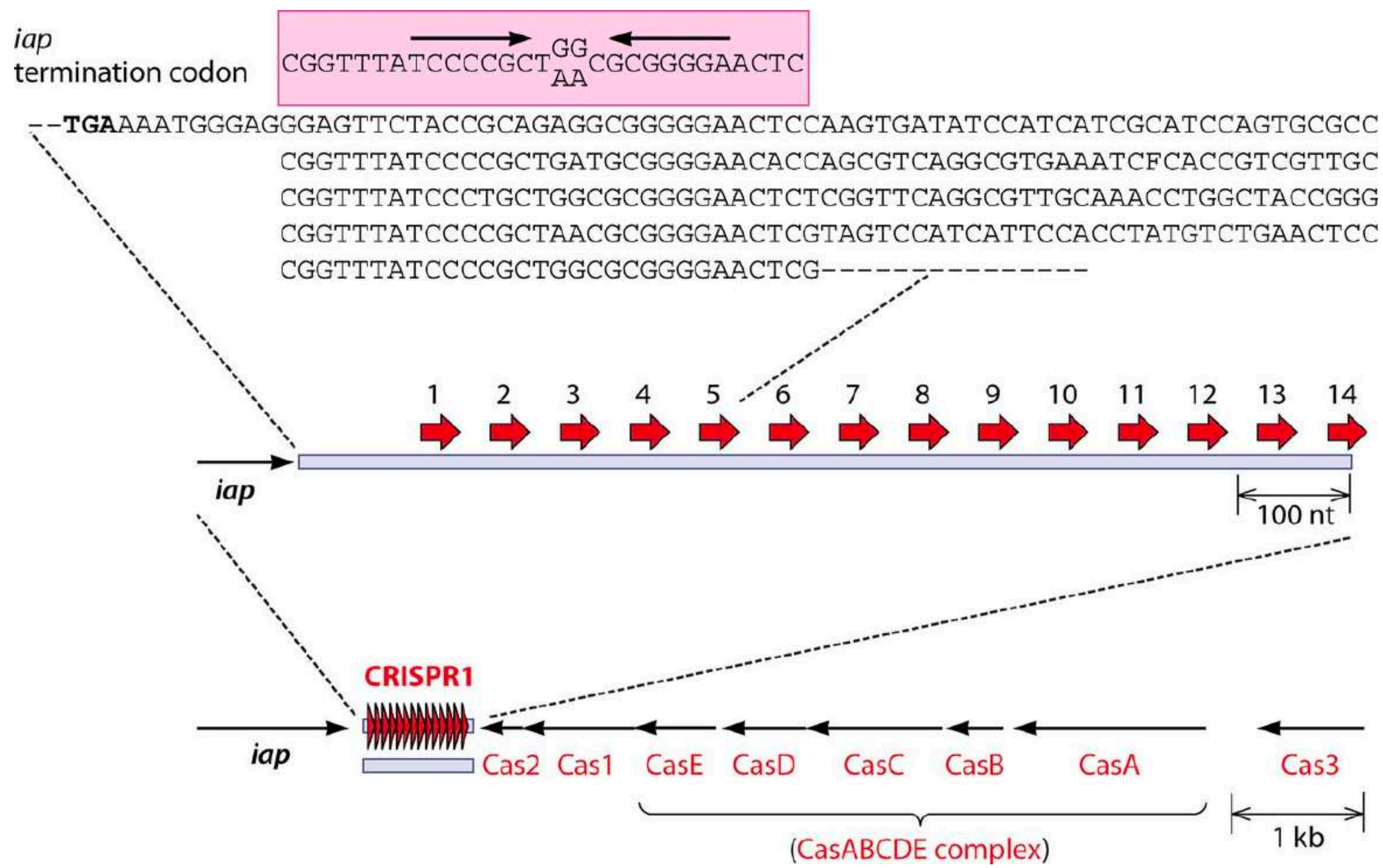
Mol. Microbiol. **2002,** 43(*6*), 1565-1575.

Protein-coding genes are associated with CRISPR arrays

Ruud Jansen

Clustered
Random
Interspersed
Short
Palindromic
Repeats

first 20-30 nt identical *next 30-40 nt always different*

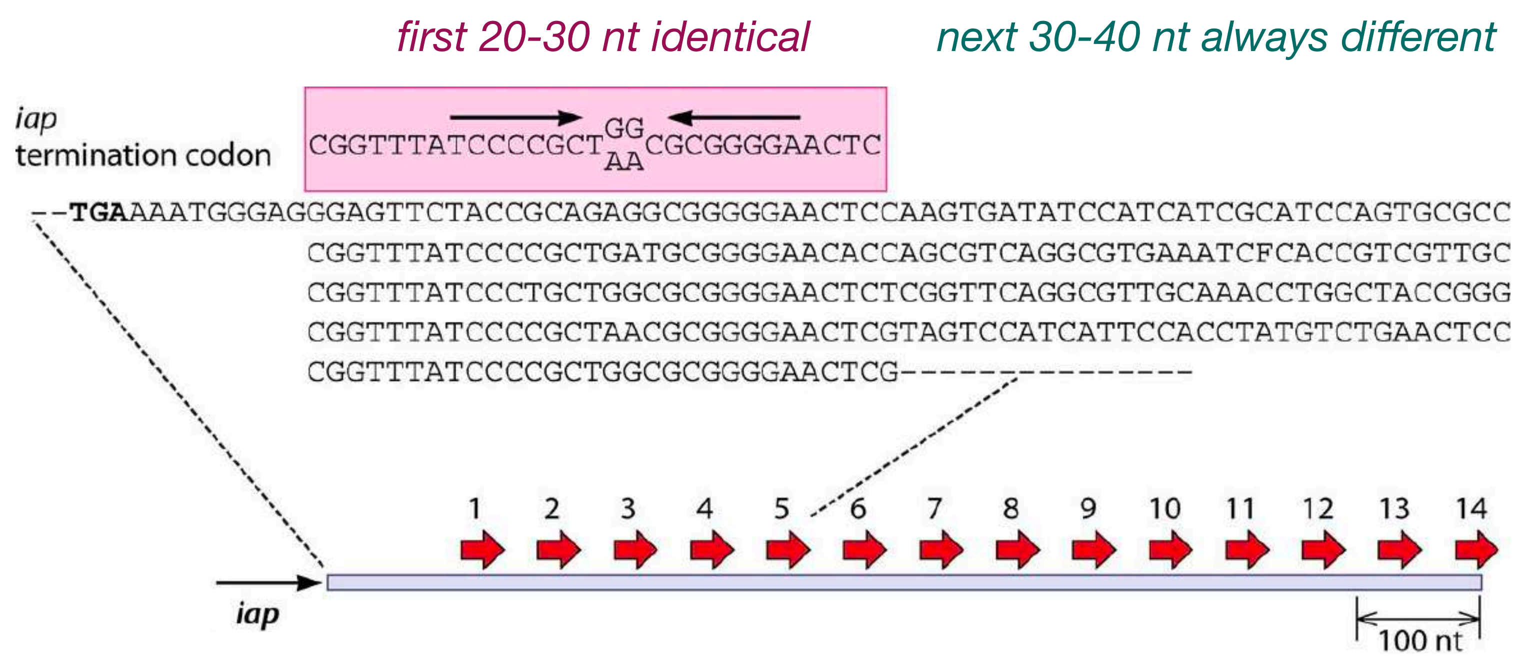


CRISPR-associated proteins (Cas)

Nucleic Acids Res. **2002,** 30(2), 482-496.

CRISPR sequences align with bacteriophage DNA

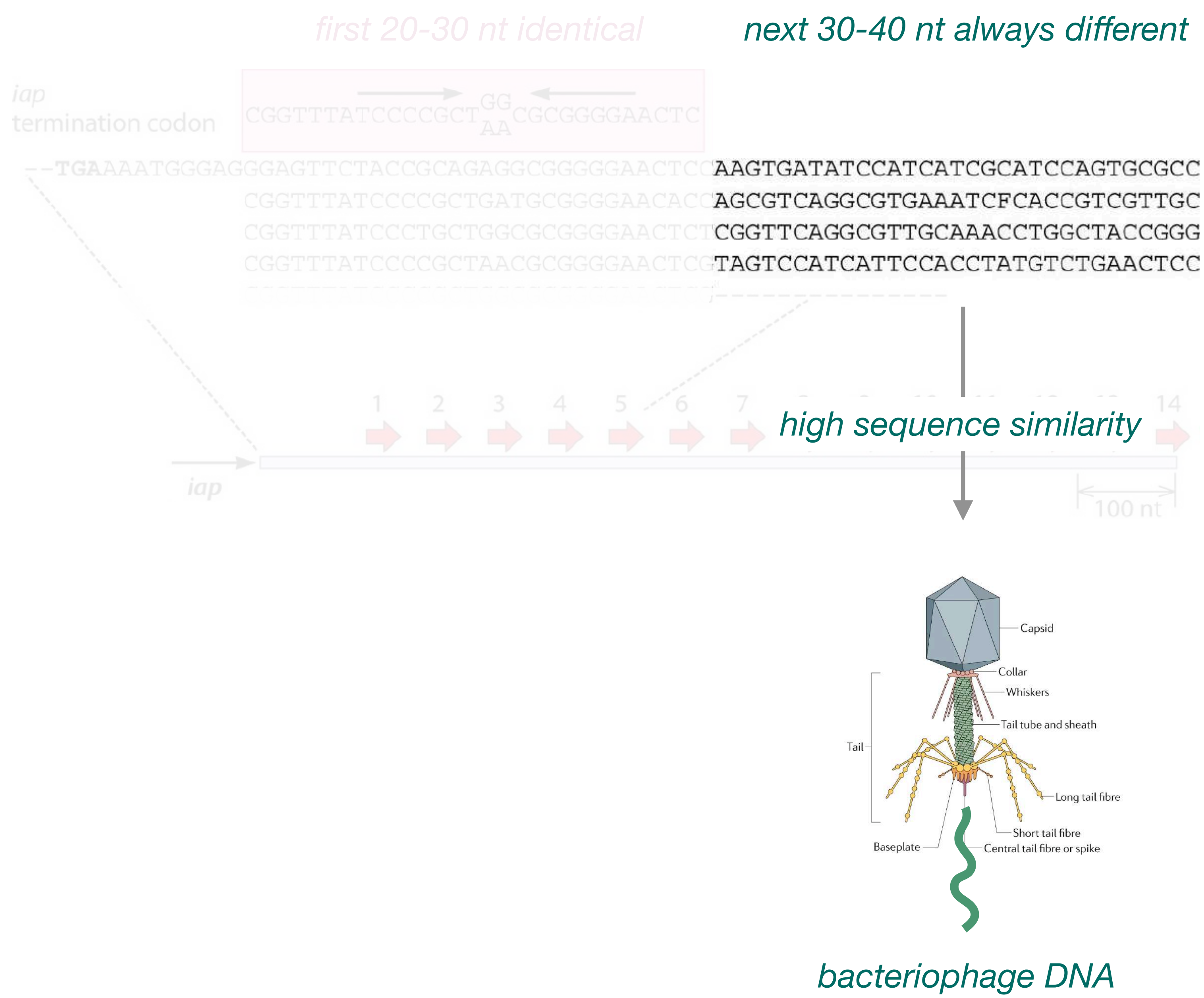
Francisco Mojica



J. Mol. Evol., **2005,** 60, 174-182.

CRISPR sequences align with bacteriophage DNA

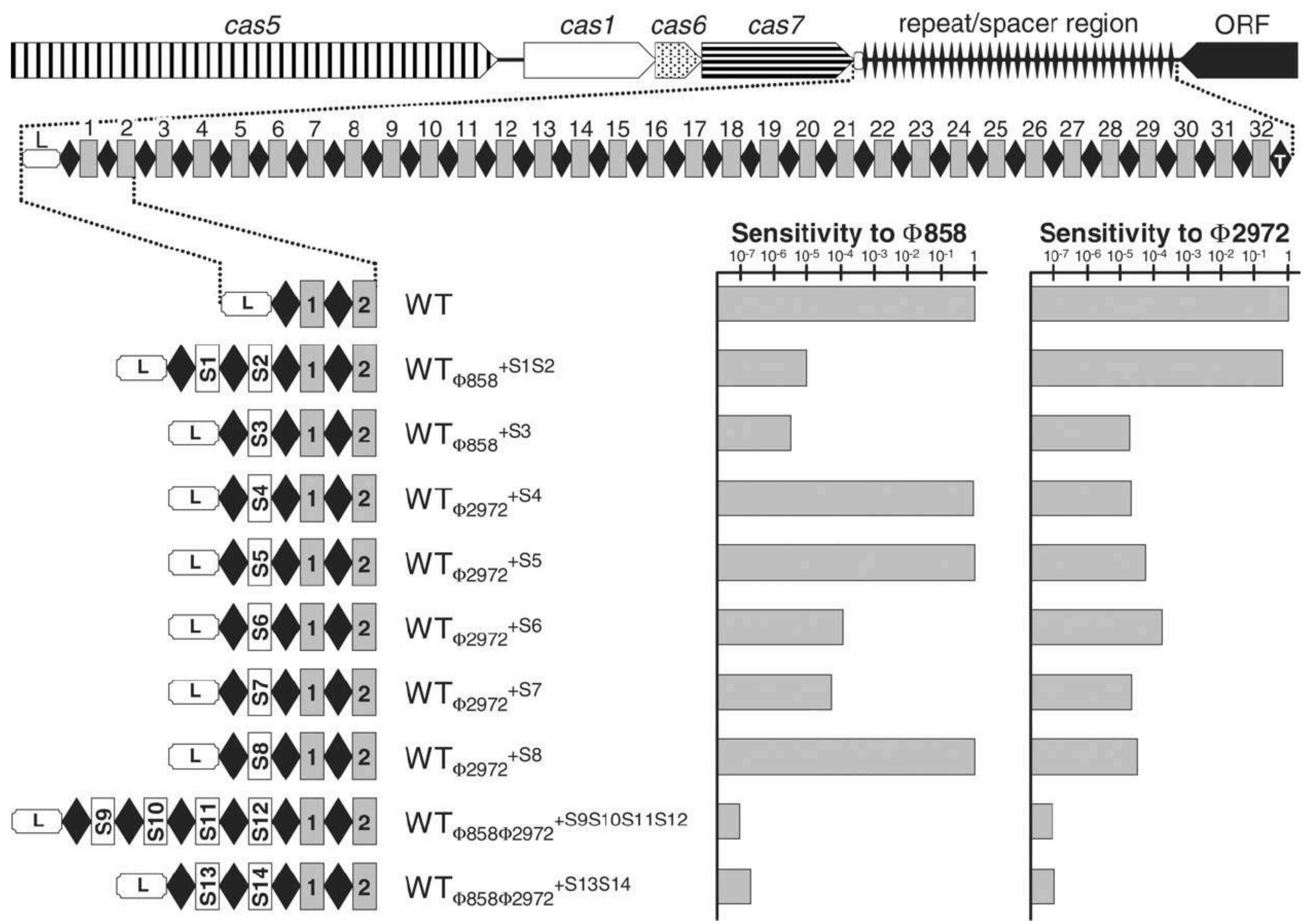
Francisco Mojica



J. Mol. Evol., **2005,** 60, 174-182.

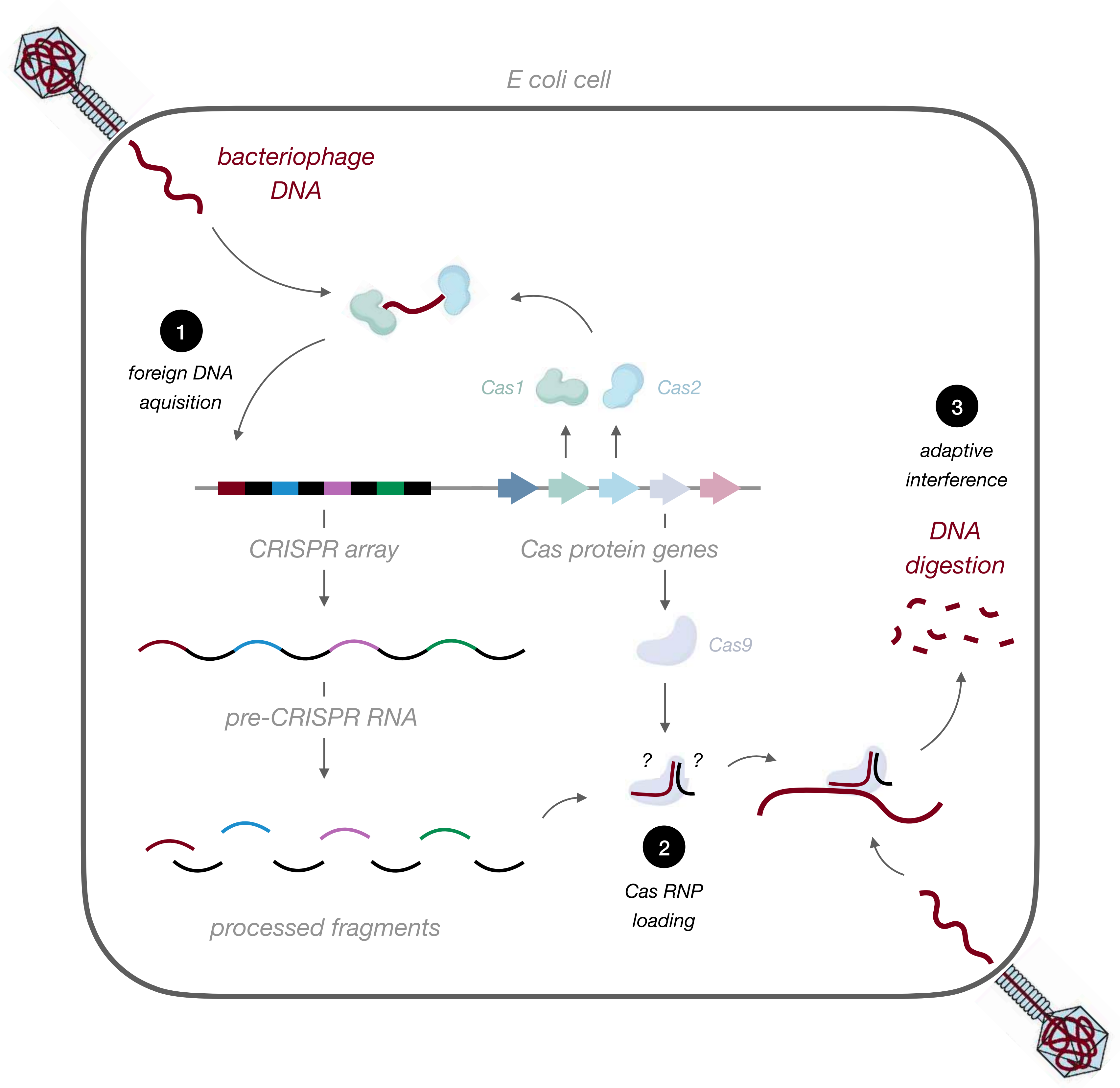
CRISPR sequences protect bacteria from infection

Rodolphe Barrangou



Science, **2007,** 315(*5819*), 1709-1712.

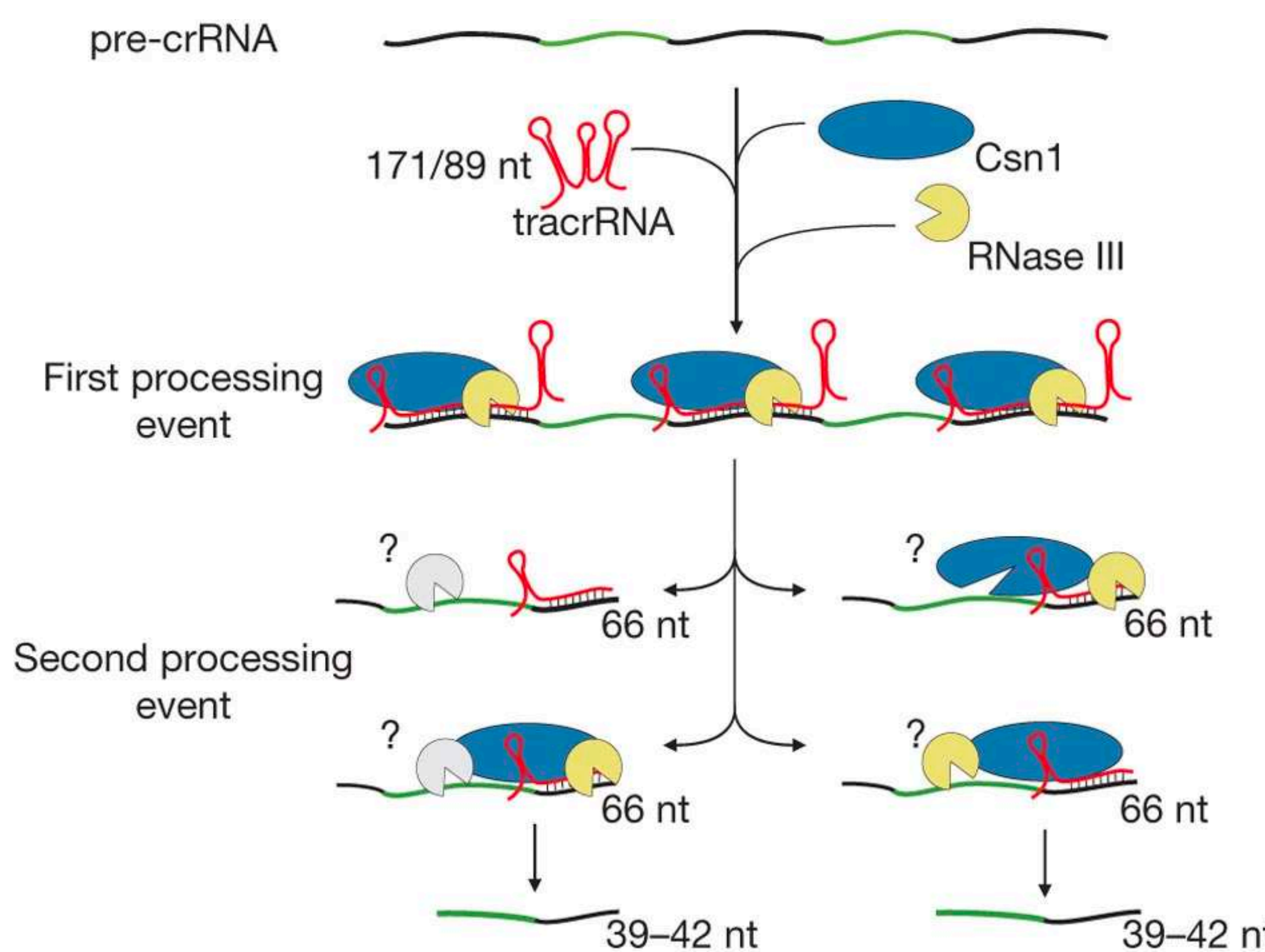
CRISPR + Cas = an adaptive antiviral immune system



Science, **2008,** 321(*5891*), 960-964.

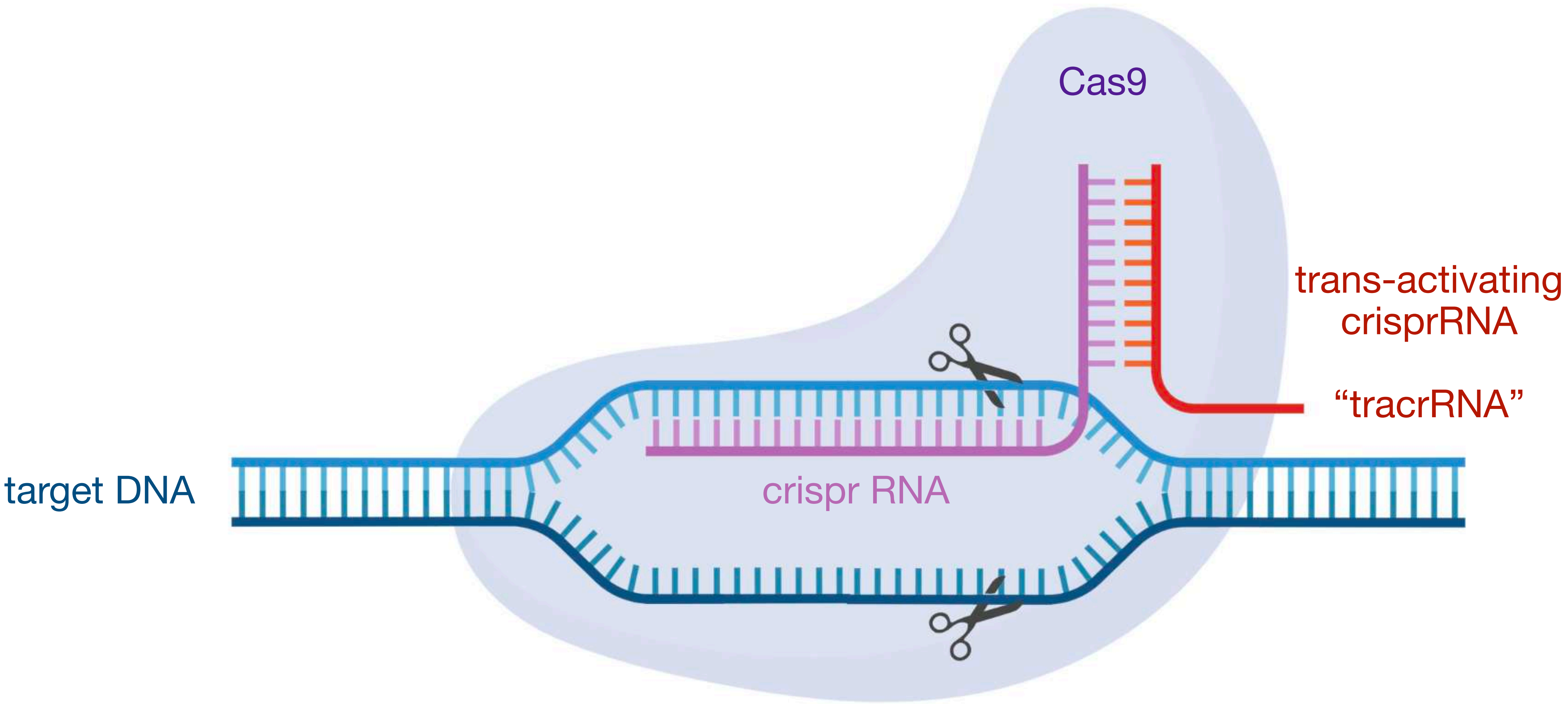
Elucidating the CRISPR-Cas architecture

Emmanuel Charpentier



Nature, **2011,** 471, 602-607.

Elucidating the CRISPR-Cas architecture

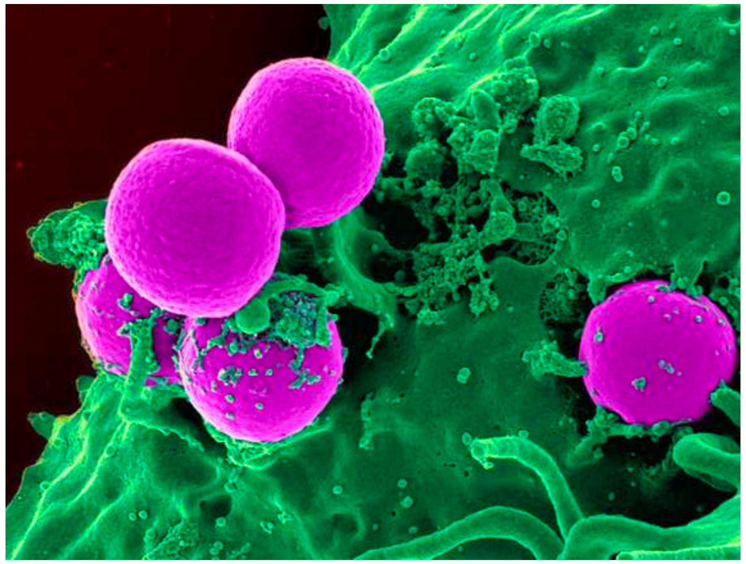


Nature, **2011,** 471, 602-607.

Later that year, in San Juan Puerto Rico

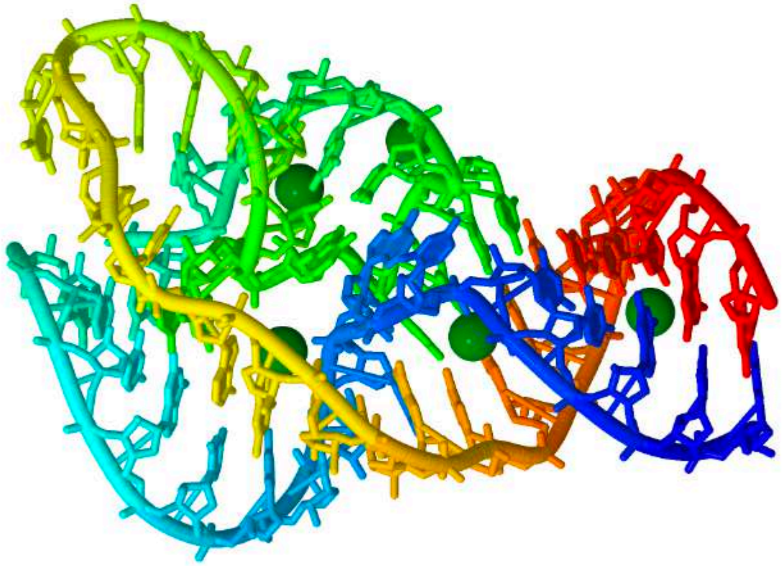
Emmanuel Charpentier

- CRISPR architecture/regulation
- inhibition of CRISPR in flesh-eating bacteria



Jennifer Doudna

- RNA-protein structural interfaces
- how does crRNA and tracrRNA function?



Science

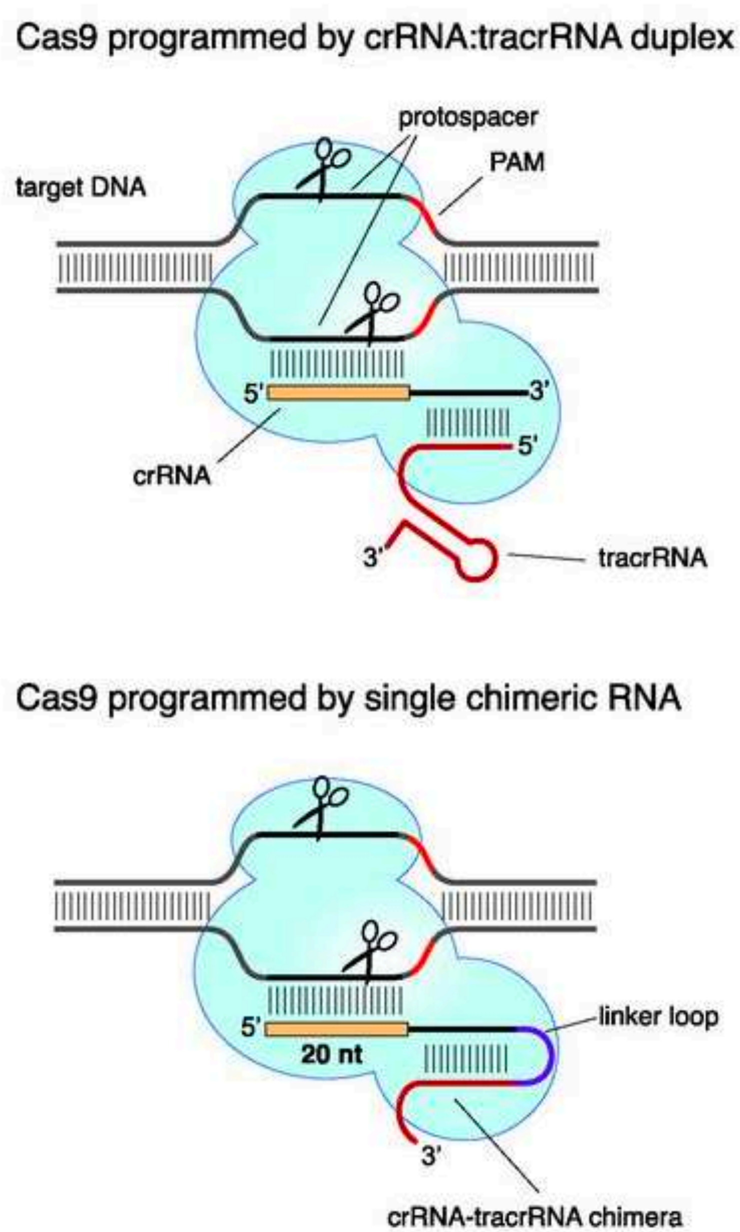
A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

17 AUGUST 2012 VOL 337 **SCIENCE** www.sciencemag.org

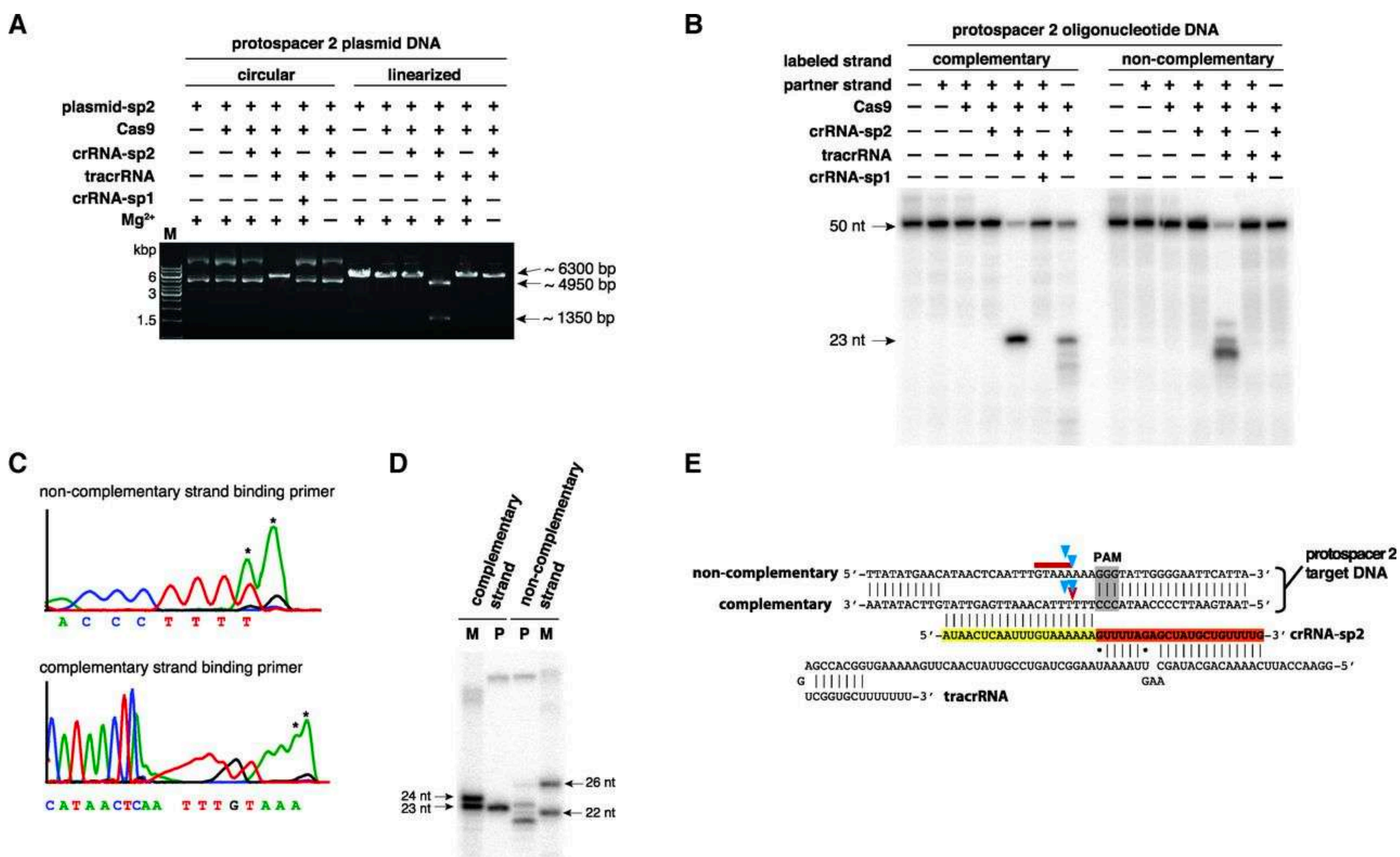
A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity



1. *better understanding of crRNA:tracrRNA interface*
2. *crRNA sequence is programmable*
3. *2 separate active sites in the Cas9 enzyme cleave each strand*
4. *fusing crRNA and tracrRNA works just as well*

Science, **2012**, 337, 816-821.

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity



Science, **2012,** 337, 816-821.

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

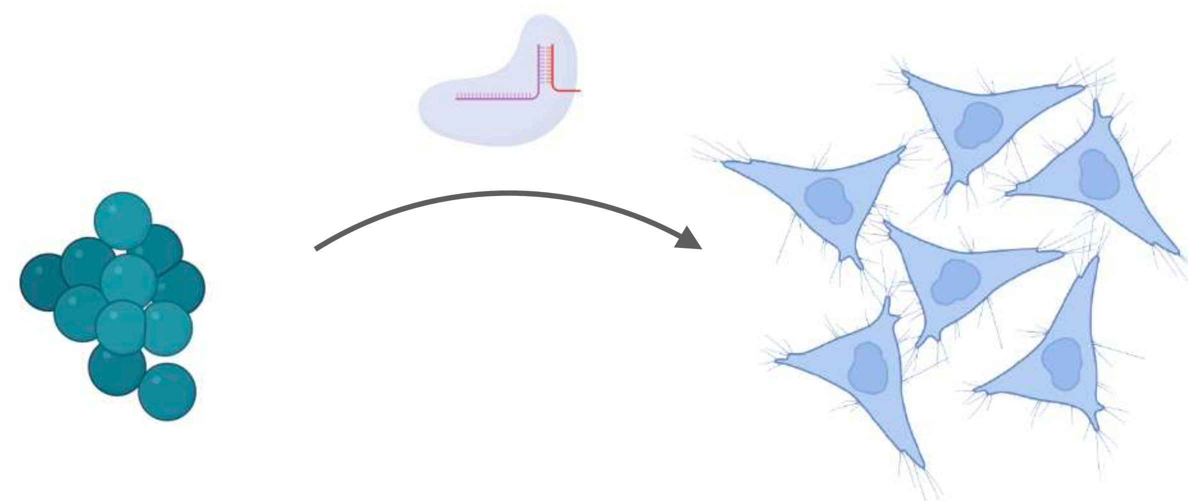
"Our study further demonstrates that the Cas9 endonuclease family can be programmed with single RNA molecules to cleave specific DNA sites, thereby raising the exciting possibility of developing a simple and versatile RNA-directed system to generate dsDNA breaks for genome targeting and editing."

"The methodology based on RNA-programmed Cas9 could offer considerable potential for gene-targeting and genome-editing applications."

Science, **2012,** 337, 816-821.

Mammalian (human) genome engineering using CRISPR Cas systems

Feng Zhang

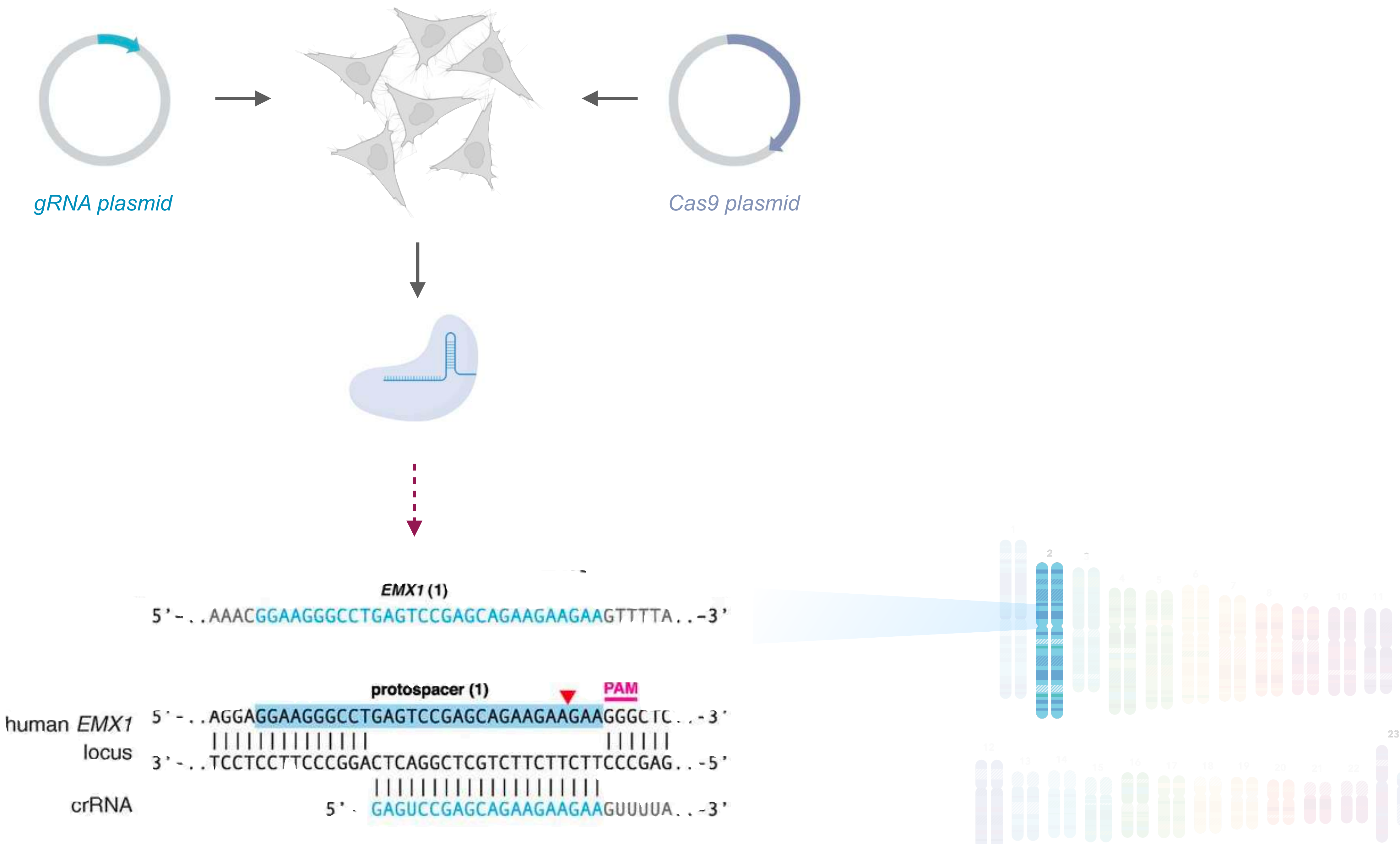


S. pyogenes

HEK293T cells

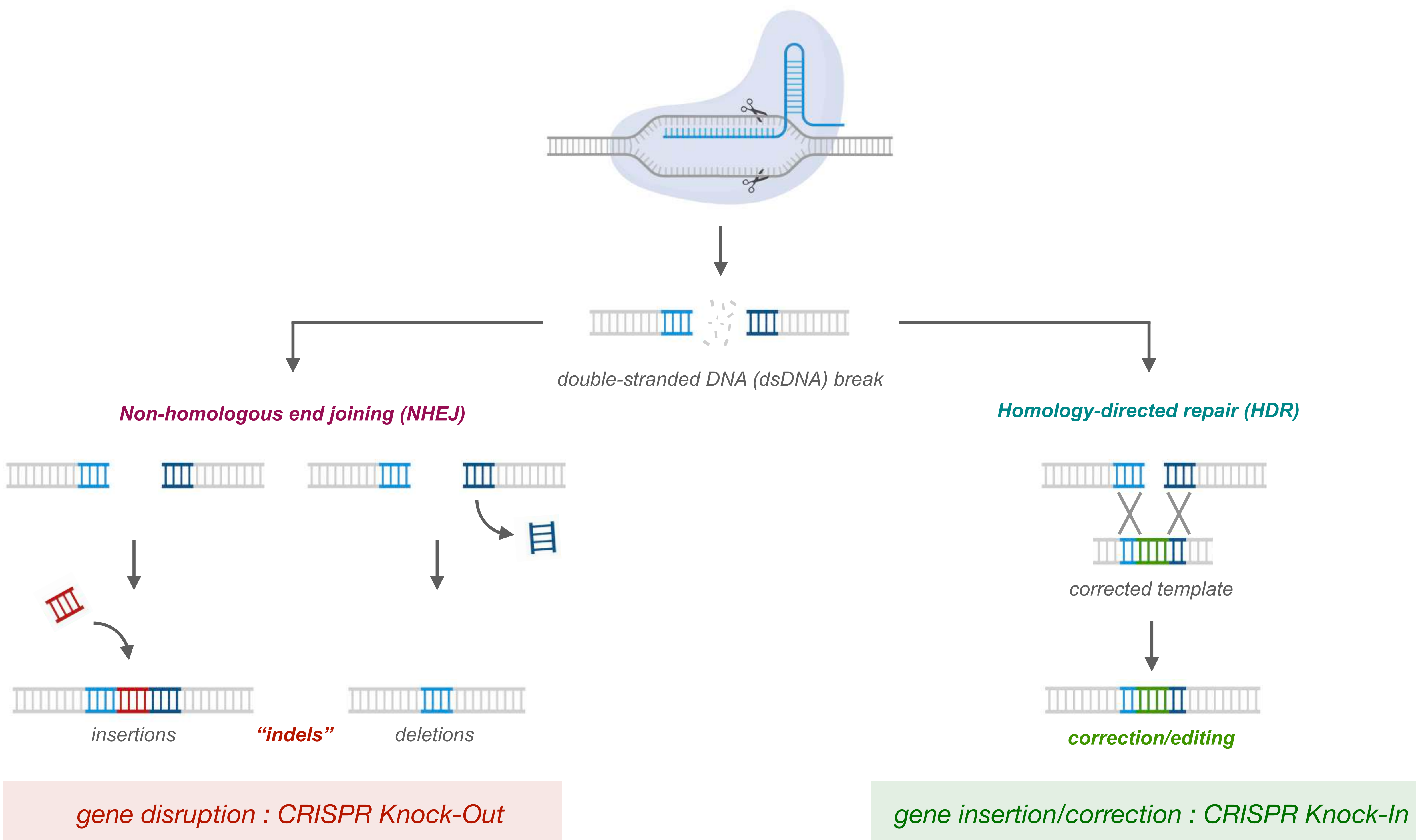
Science, **2013,** *339*(6121), 819-823.

Mammalian (human) genome engineering using CRISPR Cas systems



Science, **2013,** *339*(6121), 819-823.

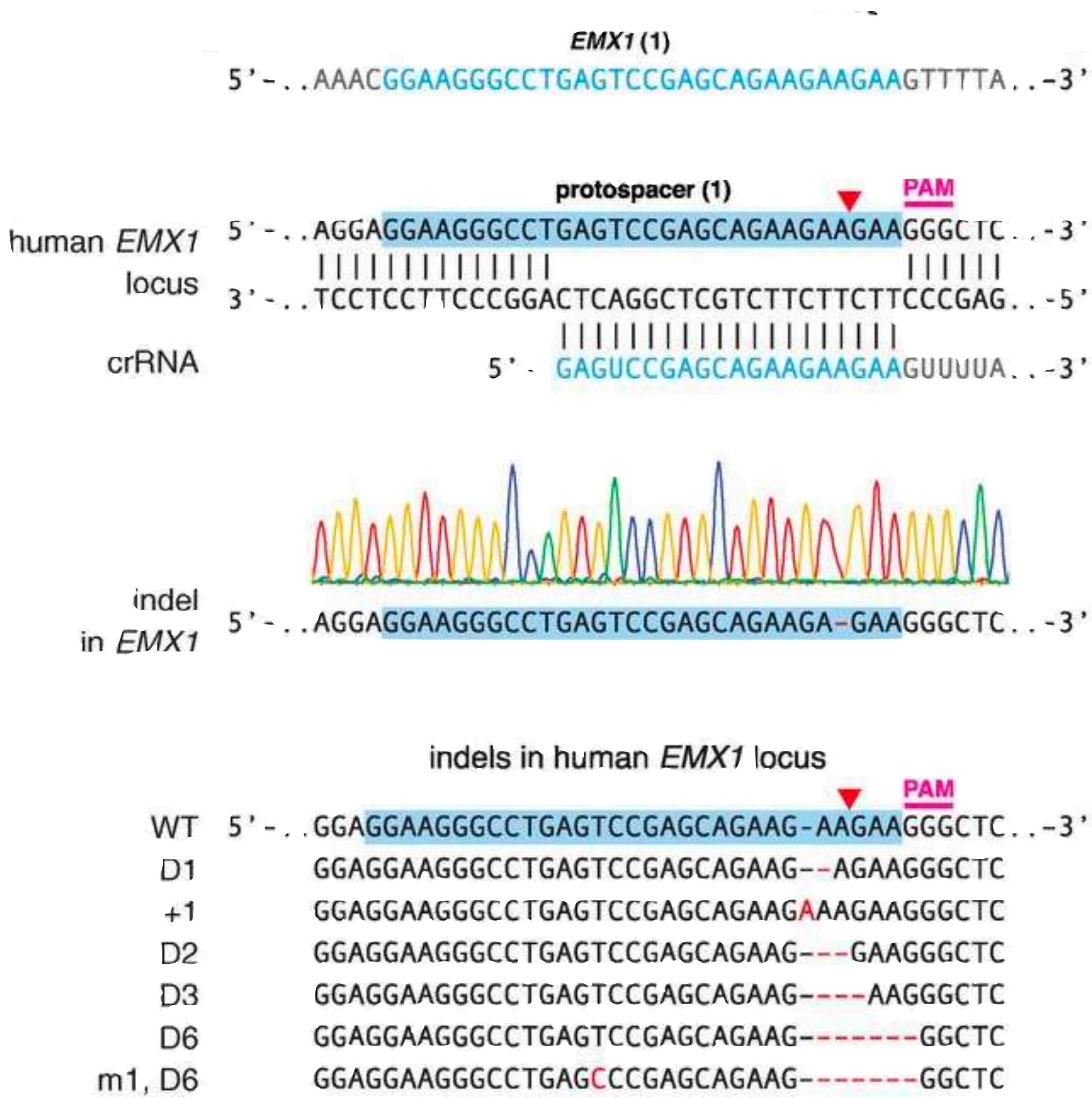
Mammalian (human) genome engineering using CRISPR Cas systems

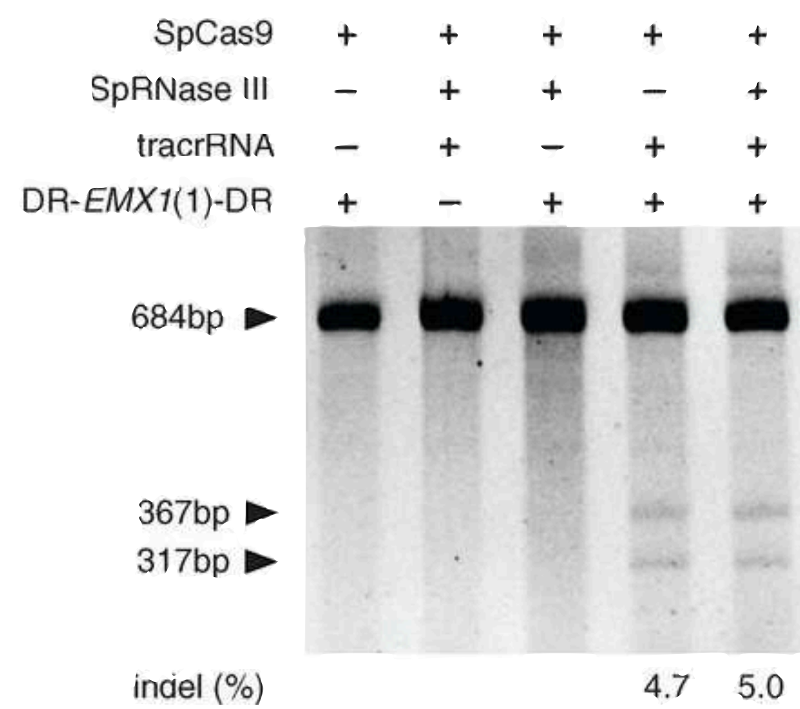


Science, **2013,** *339*(6121), 819-823.

Mammalian (human) genome engineering using CRISPR Cas systems

indel disruption

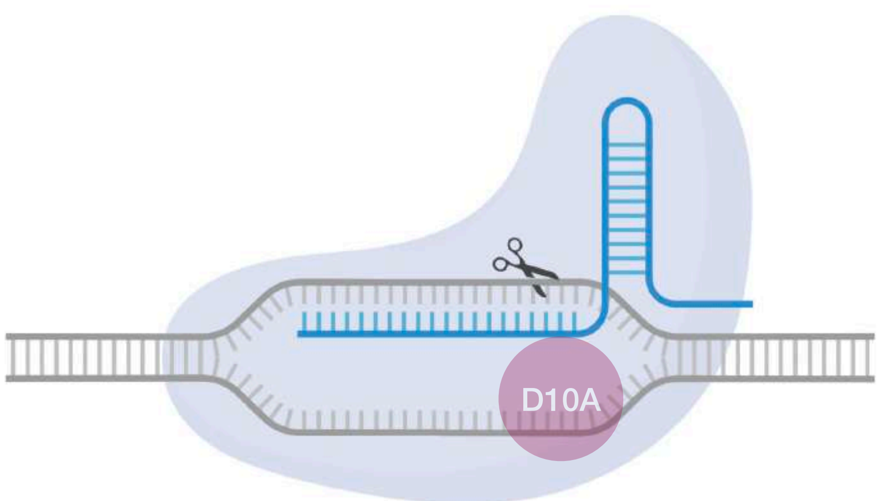


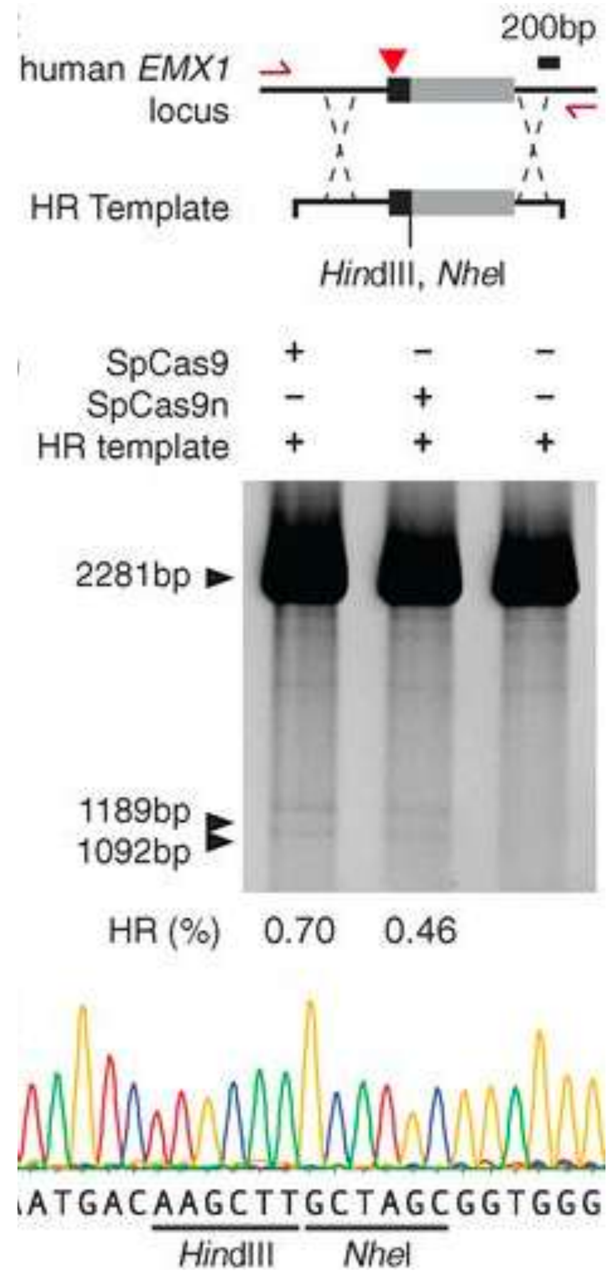


Science, **2013,** *339*(6121), 819-823.

Mammalian (human) genome engineering using CRISPR Cas systems

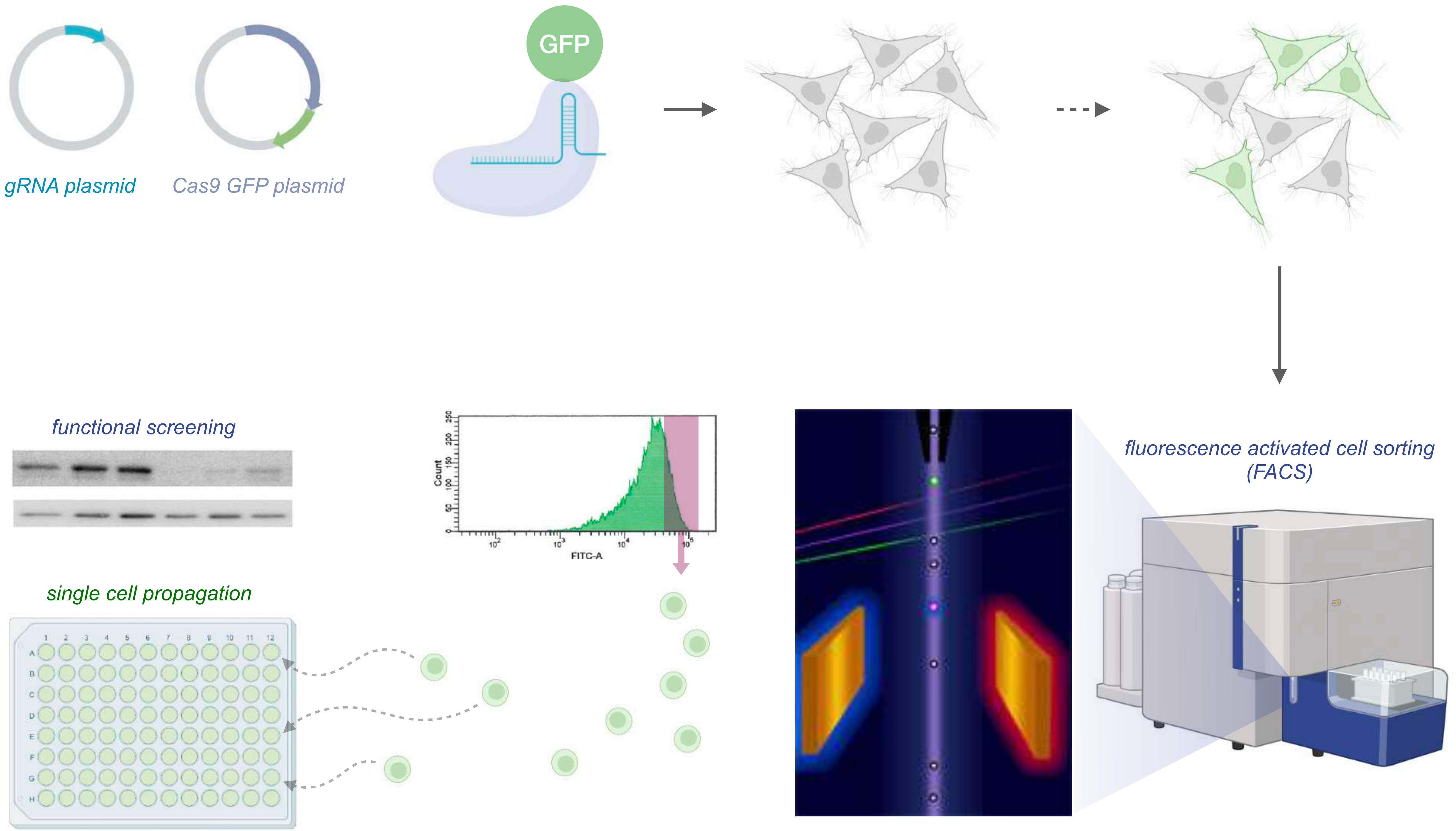
precise editing (homology-directed repair)



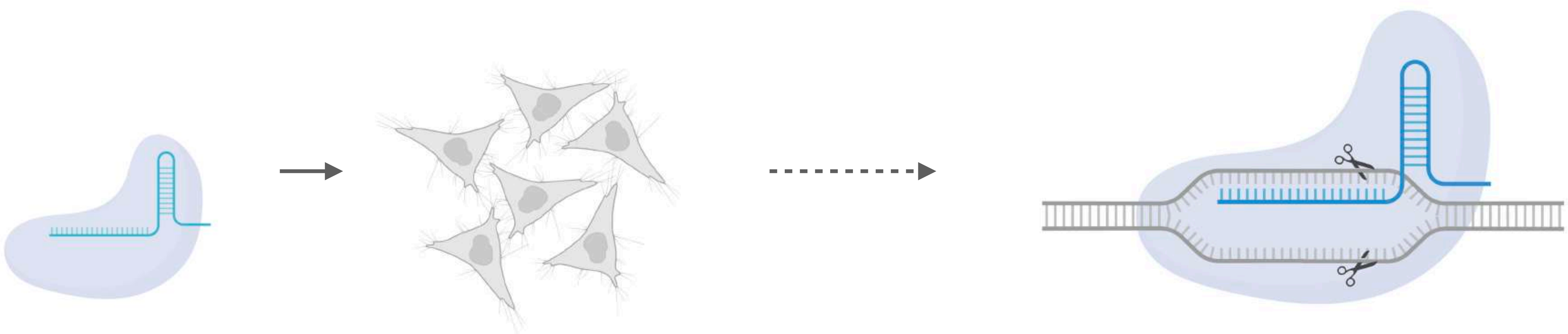


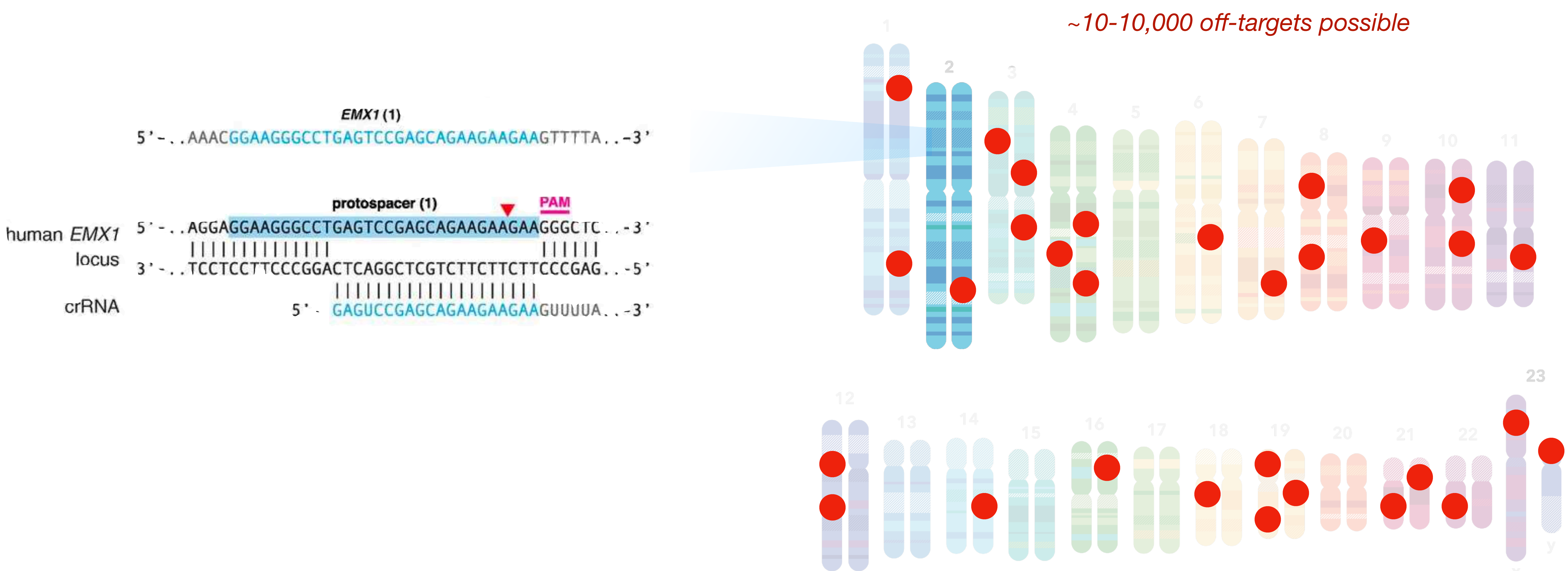
Science, **2013,** *339*(6121), 819-823.

Performing CRISPR Knock-outs and Knock-Ins



Making CRISPR Better





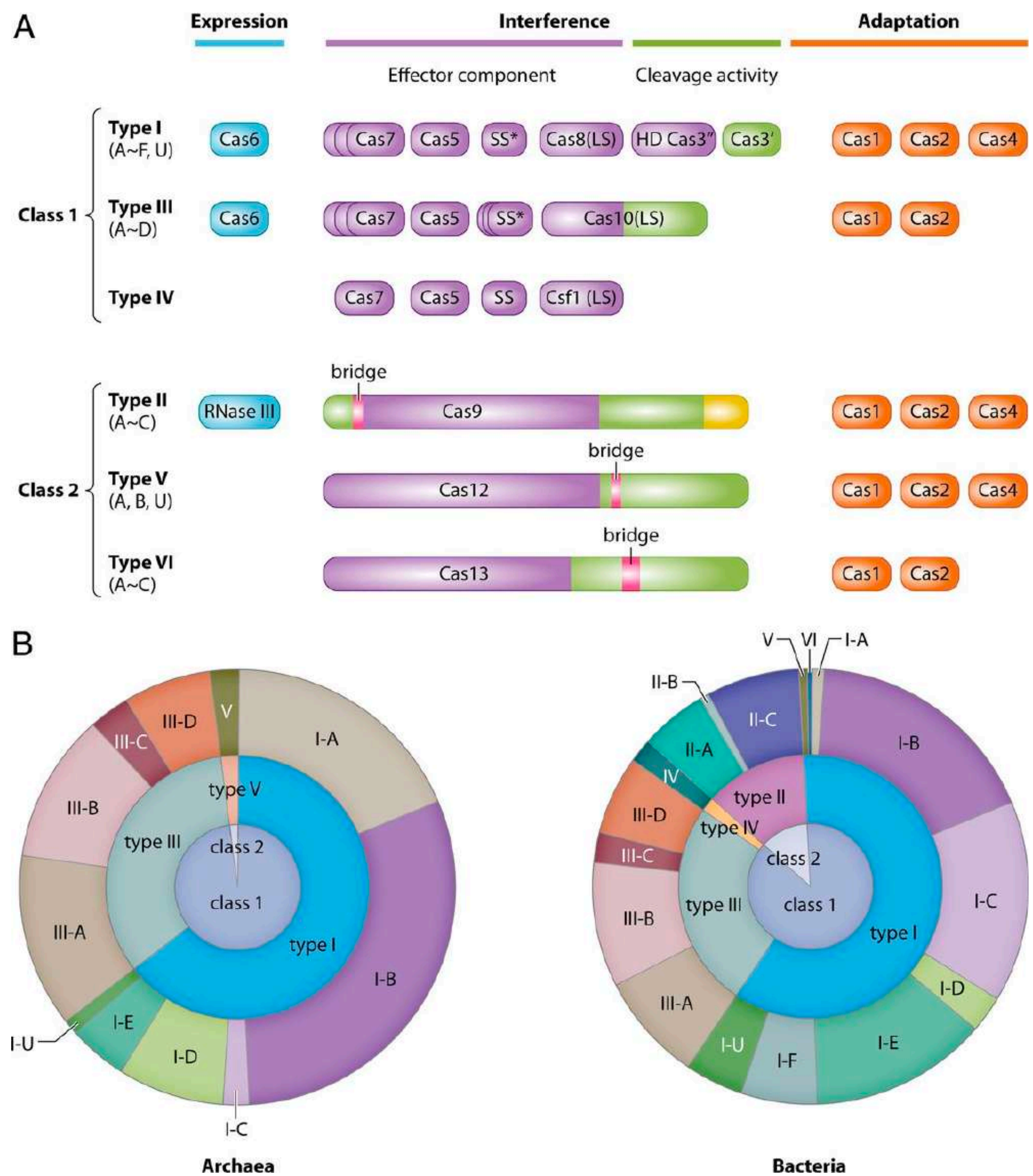
Making CRISPR Better

1. decrease off-target promiscuity

2. increase on-target efficiency

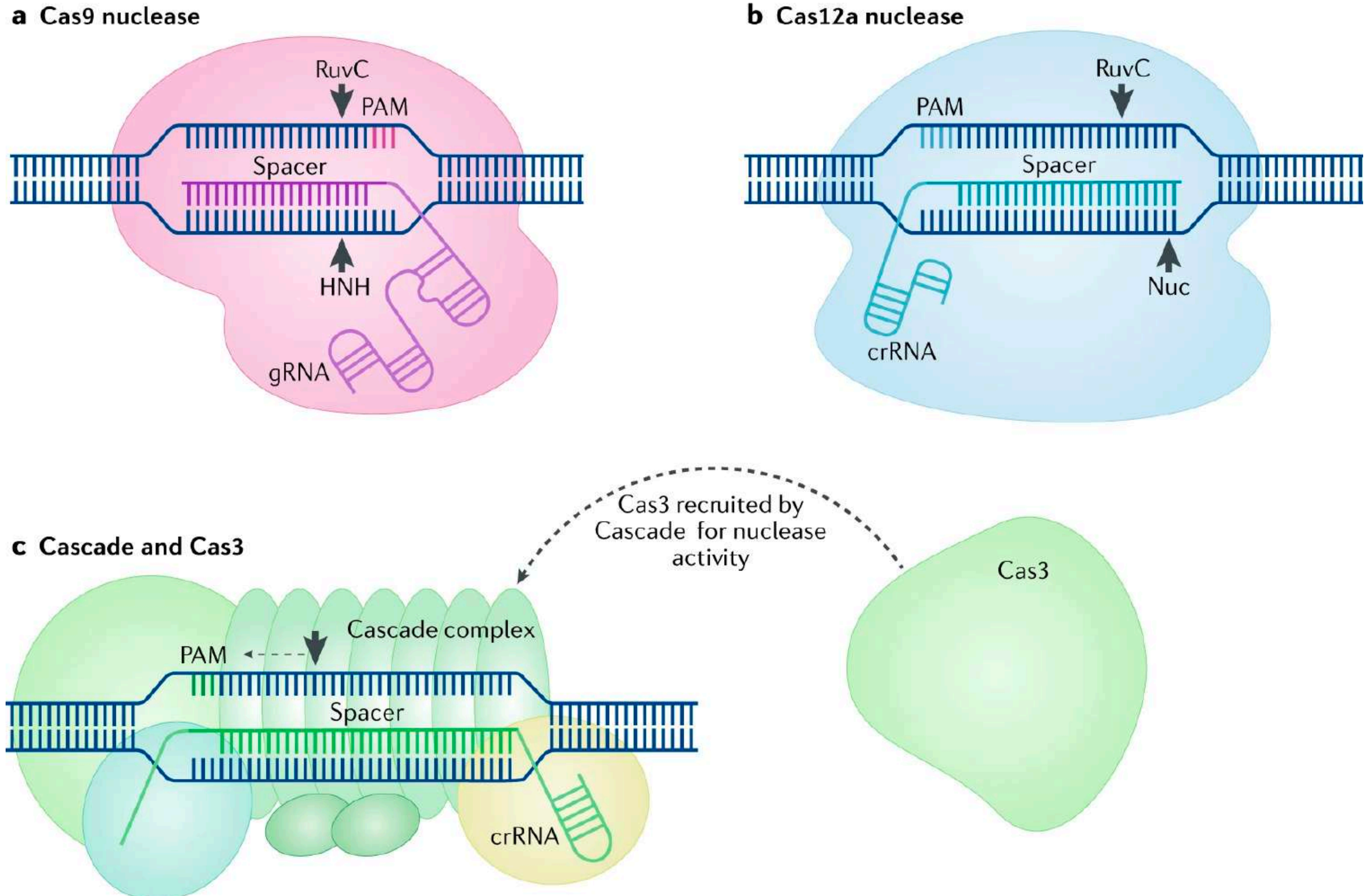
3. reduce Cas9 size/enhance “deliverability”

CRISPR Cas systems across microbiology



Curr. Opin. Microbiol., **2017,** 37, 67-68.

Natural alternatives and engineered Cas9 systems



Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

Natural alternatives and engineered Cas9 systems

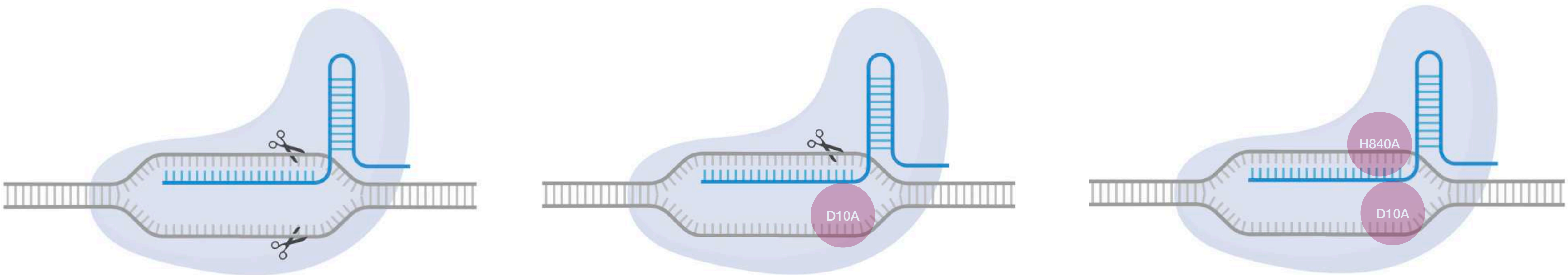
Table 1 | **Cas9 variants with altered protospacer adjacent motif and targeting specificities**

Name	Description of protein variant or mutations	PAM (5′ to 3′)	Notes
SpCas9	Native *Streptococcus pyogenes* Cas9	NGG[248]	1,368 amino acids
VRER SpCas9[a]	D1135V, G1218R, R1335E, T1337R	NGCG[17]	Altered PAM variant; bacterial selection-based screening
VQR SpCas9[b]	D1135V, R1335Q, T1337R	NGAN or NGNG[17]	Altered PAM variant; bacterial selection-based screening
EQR SpCas9[b]	D1135E, R1335Q, T1337R	NGAG[17]	Altered PAM variant; bacterial selection-based screening
xCas9-3.7[c]	A262T, R324L, S409I, E480K, E543D, M694I, E1219V	NG, GAA, GAT[18]	Altered PAM variant; phage-assisted continuous evolution
eSpCas9 (1.0)[d]	K810A, K1003A, R1060A	NGG	Enhanced specificity; structure-guided protein engineering[19]
eSpCas9 (1.1)[d]	K810A, K1003A, R1060A	NGG	Enhanced specificity; structure-guided protein engineering[19]
Cas9-HF1[e]	N497A, R661A, Q695A, Q926A	NGG	Enhanced specificity[20]
HypaCas9[f]	N692A, M694A, Q695A, H698A	NGG	Enhanced specificity[21]
evoCas9[g]	M495V, Y515N, K526E, R661Q	NGG	Enhanced specificity; yeast-based screening[22]
HiFi Cas9[e]	R691A	NGG	Enhanced specificity for ribonucleoprotein delivery[23]
ScCas9	Native *Streptococcus canis* Cas9	NNG[249]	1,375 amino acids
StCas9	Native *Streptococcus thermophilus* Cas9	NNAGAAW[11,25]	1,121 amino acids
NmCas9	Native *Neisseria meningitidis* Cas9	NNNNGATT[26–28]	1,082 amino acids
SaCas9	Native *Staphylococcus aureus* Cas9	NNGRRT[29]	1,053 amino acids
CjCas9	Native *Campylobacter jejuni* Cas9	NNNVRYM[30]	984 amino acids
CasX	Phyla Deltaproteobacteria and Planctomycetes	TTCN[32]	980 amino acids

PAM, protospacer adjacent motif. [a]*S. pyogenes* Cas9 variant with quadruple mutations; [b]*S. pyogenes* Cas9 variant with triple mutations; [c]expanded PAM *S. pyogenes* Cas9 variant; [d]enhanced-specificity *S. pyogenes* Cas9 variant; [e]high-fidelity Cas9 variant; [f]hyperaccurate Cas9 variant; [g]evolved high-fidelity Cas9 variant.

Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

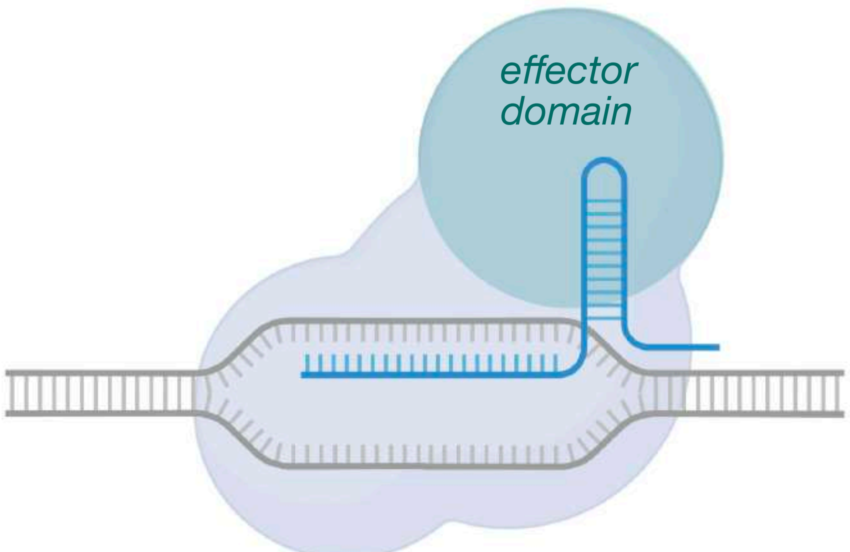
Killing Cas



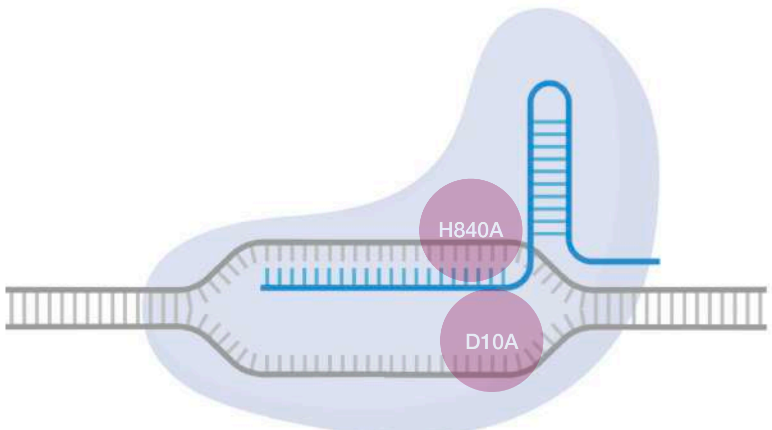
Full Cas9 endonuclease | Cas9 *"nickase"* | ***dead Cas9***

Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

Bringing new life to new activity



dead Cas9 - fusions…



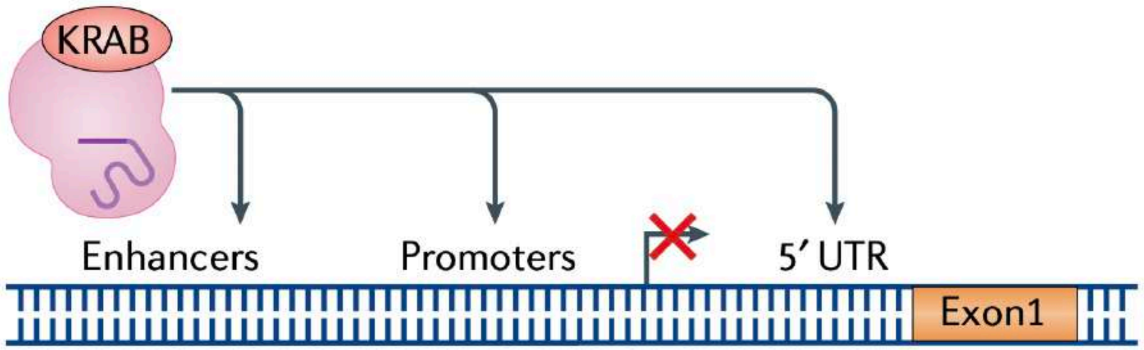
dead Cas9

Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

Epigenetic regulation of gene expression

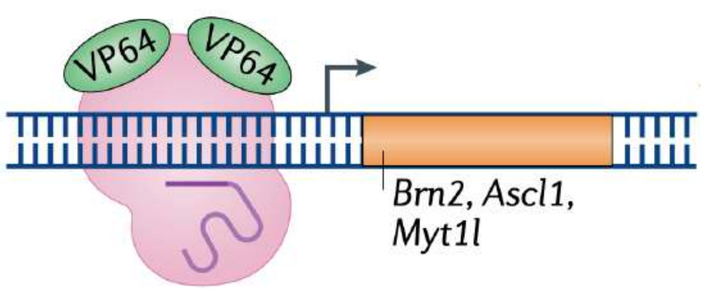
CRISPRi

inhibit gene expression



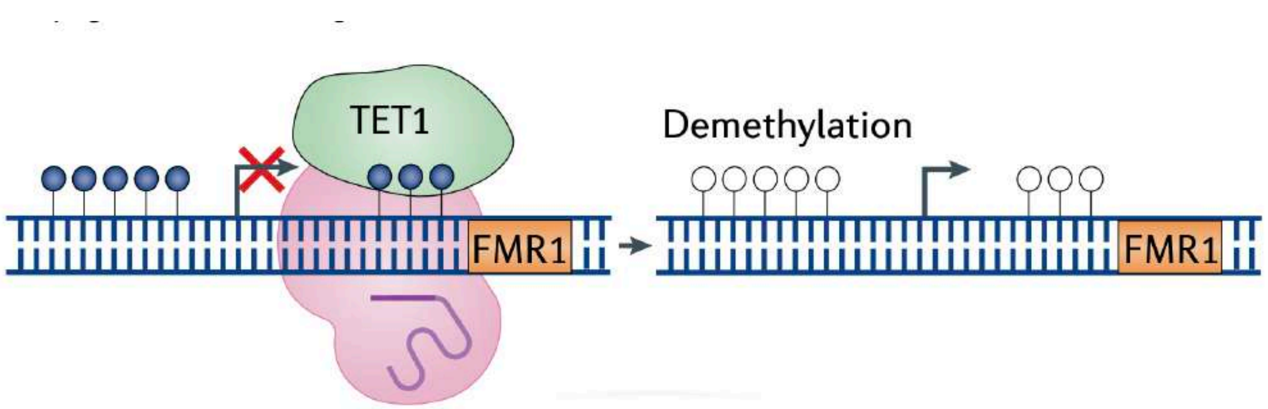
CRISPRa

activate gene expression



chromatin remodeling

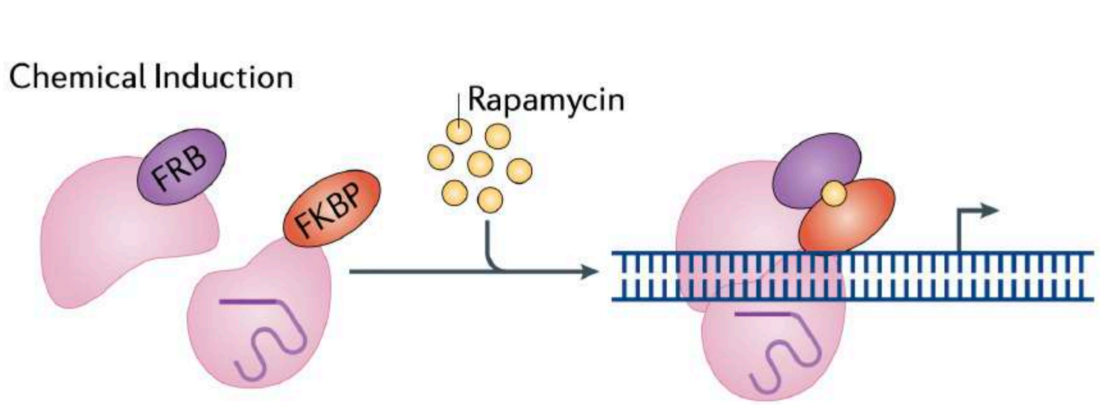
methylation, acetylation, removal, installation, etc

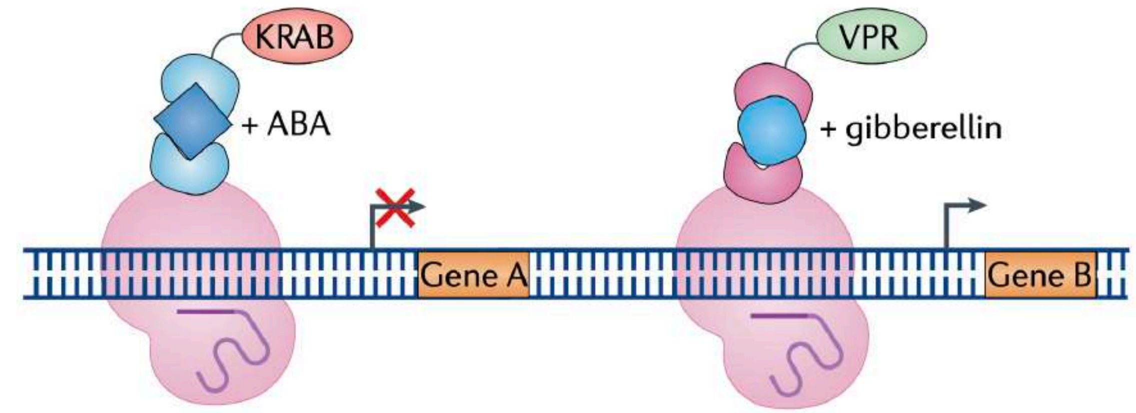


Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

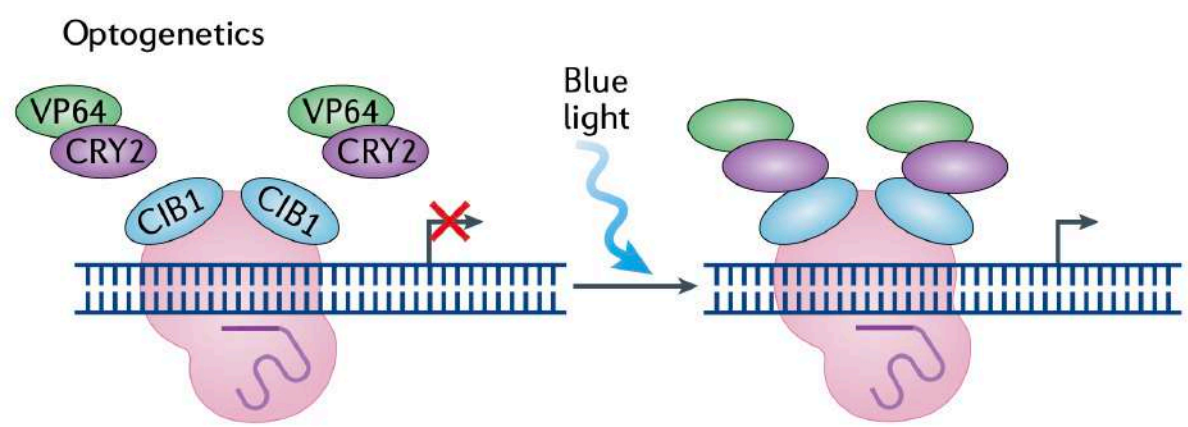
Epigenetic regulation of gene expression

small molecule-induced dimerization



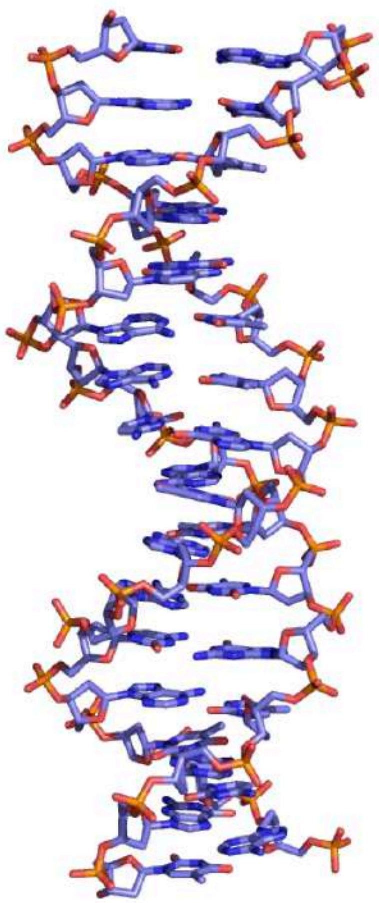


light-based recruitment

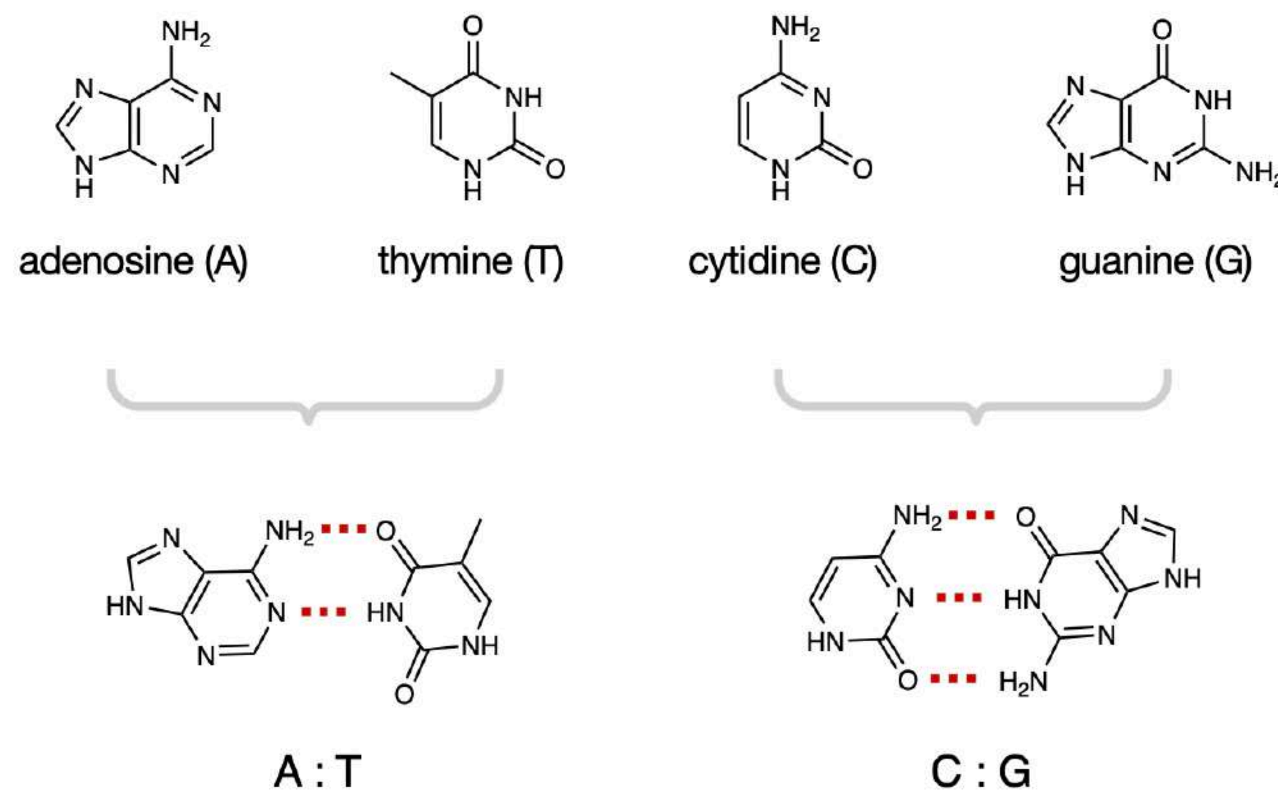


Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

Base editing



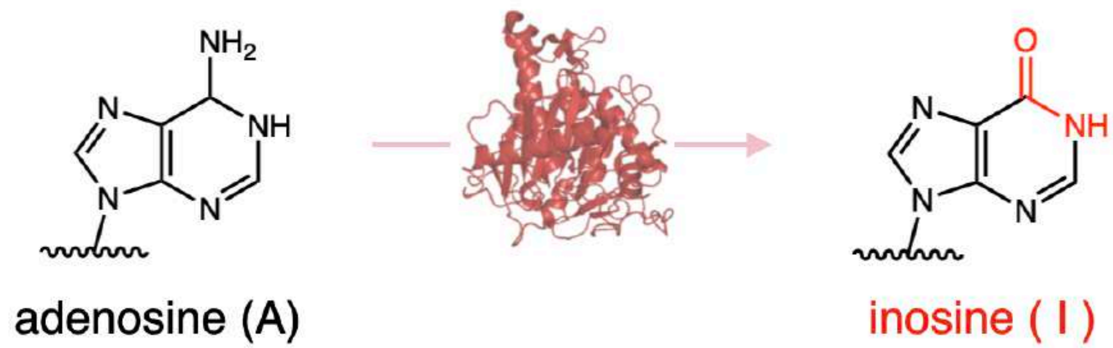
DNA

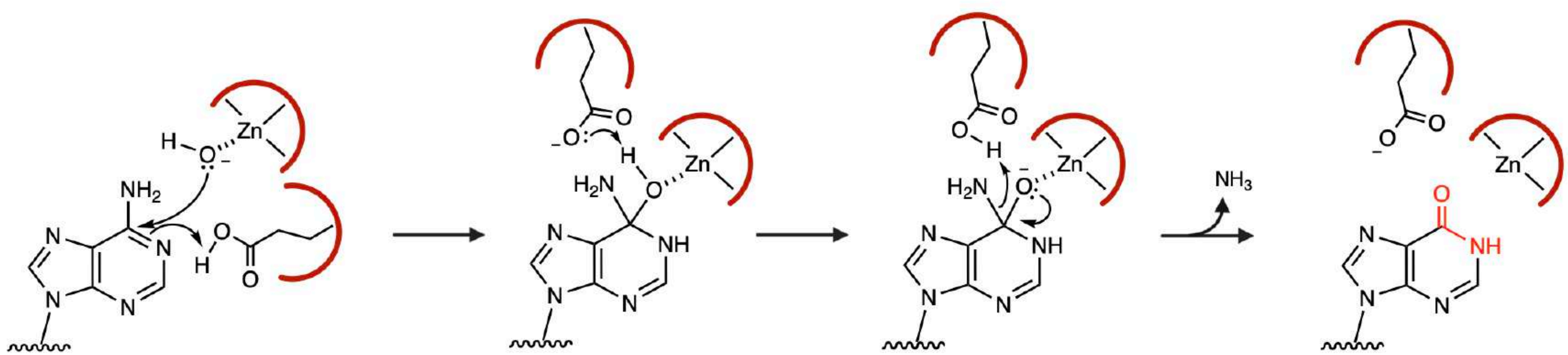


Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

Base editing

adenosine deamination

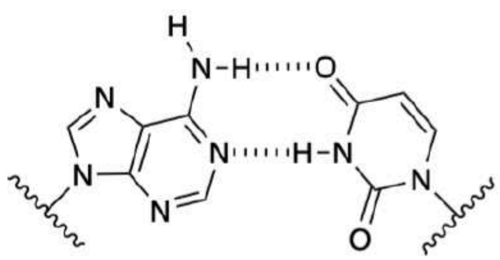




Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

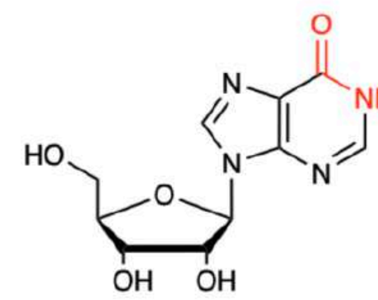
Base editing

adenosine deamination



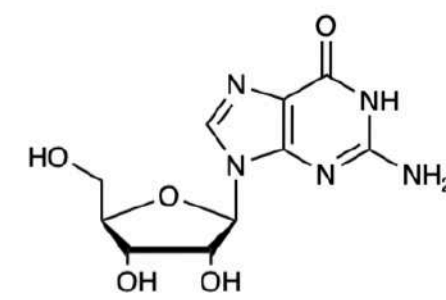
adenosine : uracil basepairing

inosine : cytidine basepairing



inosine (I)

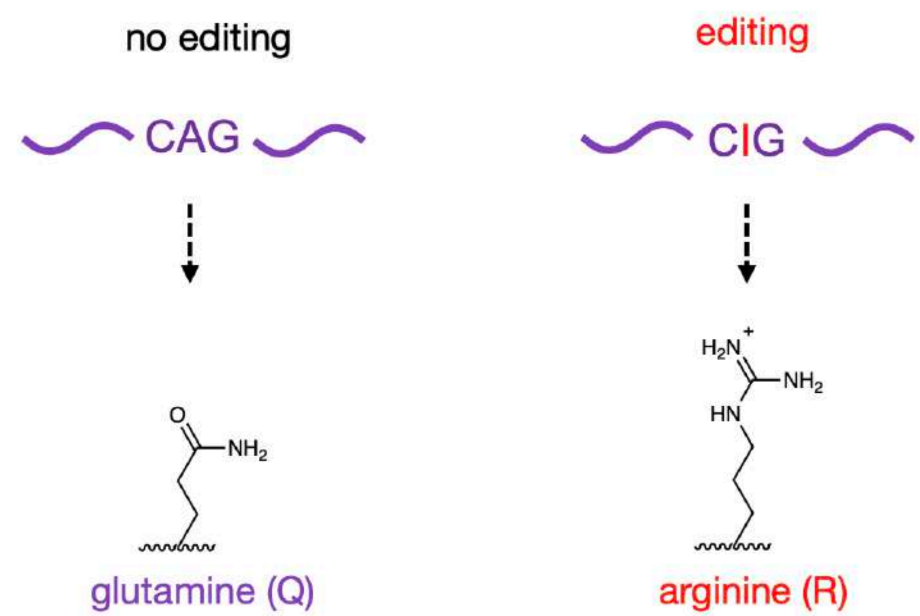
≈



guanosine (G)

Second letter (A → G)

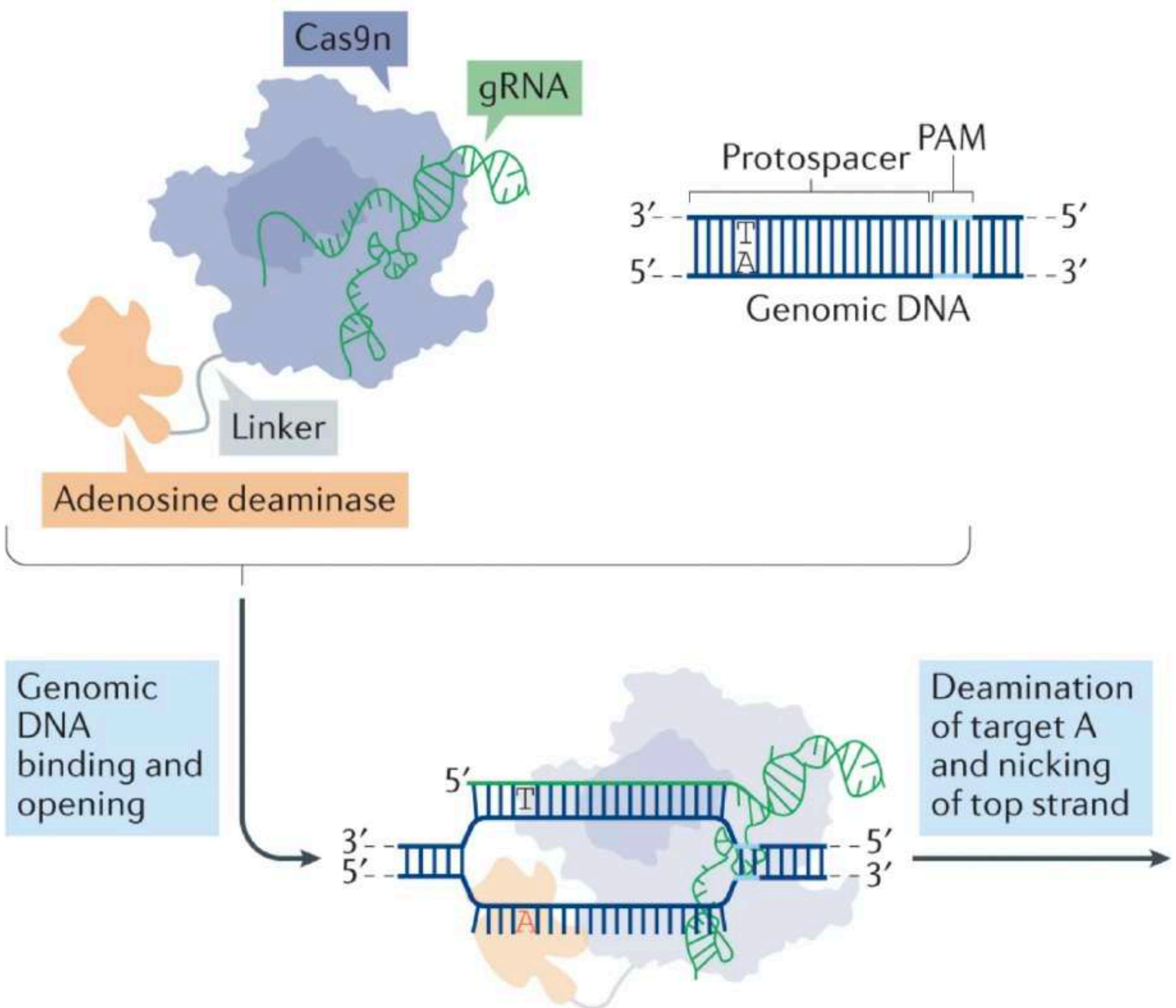
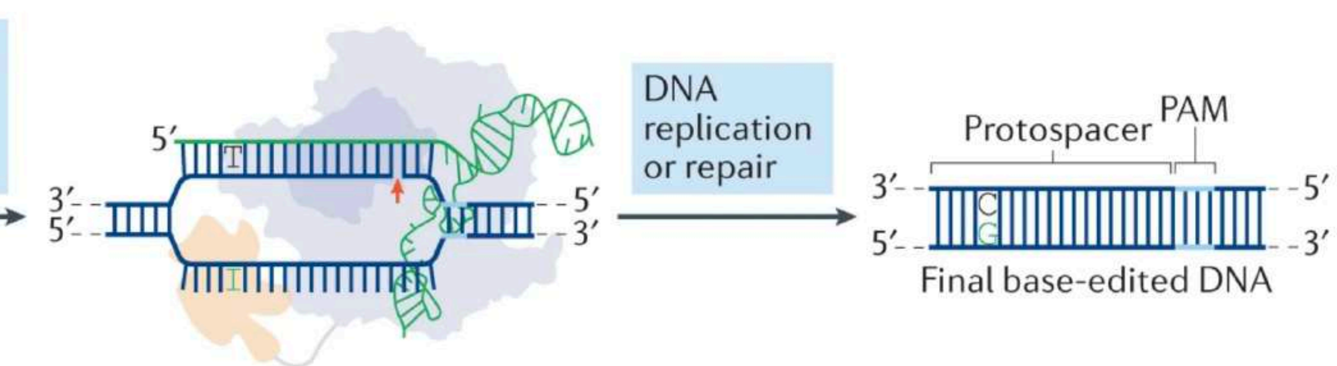
First letter	U	C	A	G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
U	UUC	UCC	UAC	UGC	C
U	UUA Leu	UCA	UAA Stop	UGA Stop	A
U	UUG	UCG	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
C	CUC	CCC	CAC	CGC	C
C	CUA	CCA	CAA Gln	CGA	A
C	CUG	CCG	CAG	CGG	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
A	AUC	ACC	AAC	AGC	C
A	AUA	ACA	AAA Lys	AGA Arg	A
A	AUG Met	ACG	AAG	AGG	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
G	GUC	GCC	GAC	GGC	C
G	GUA	GCA	GAA Glu	GGA	A
G	GUG	GCG	GAG	GGG	G



Nat. Rev. Mol. Cell. Biol., **2019,** *20*, 490-507.

Base editing

adenosine deamination

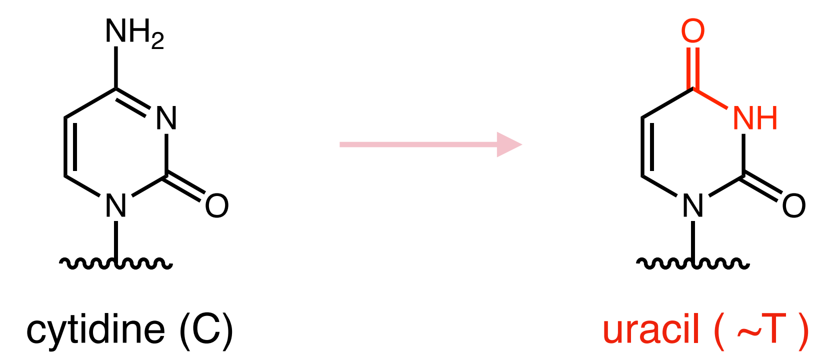
Alexis Komor

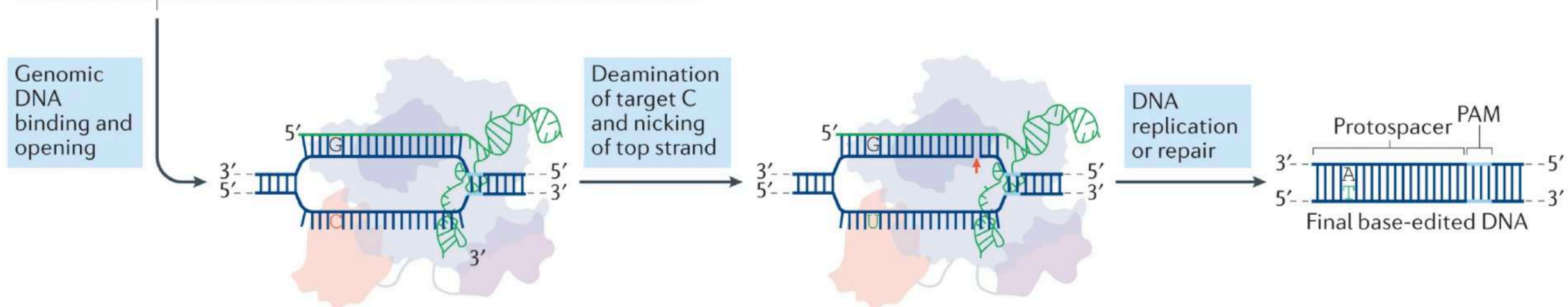
David Liu

Nature, **2016,** *533*, 420-424.

Base editing

cytidine deamination

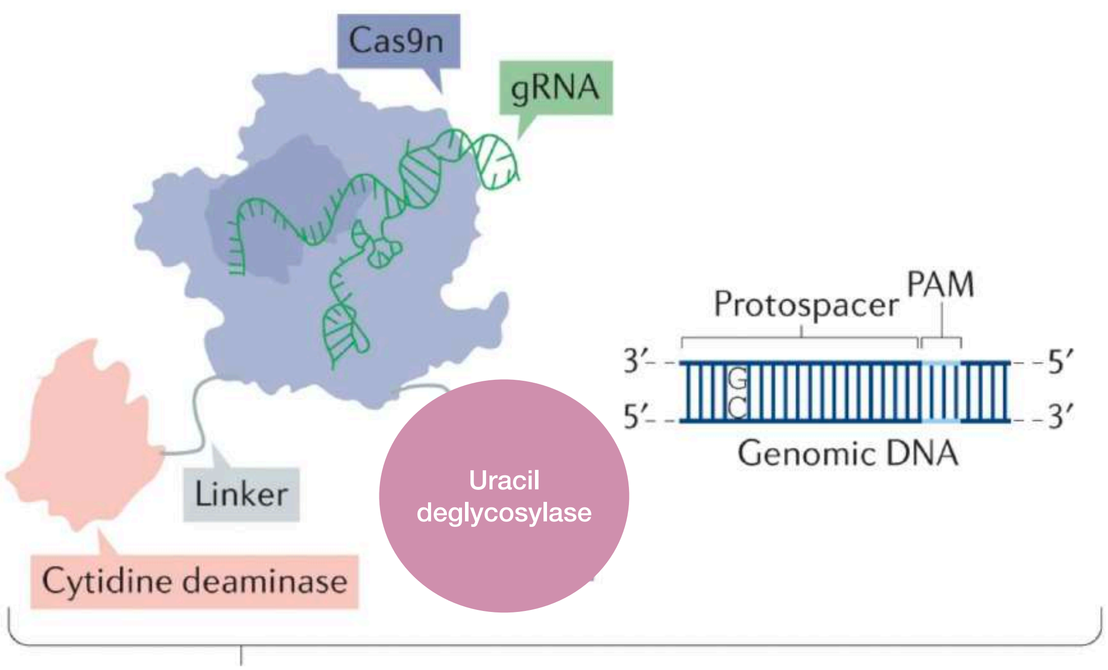


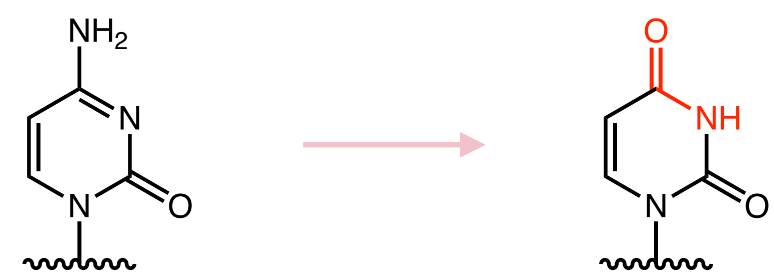


Nature, **2016,** *533*, 420-424.

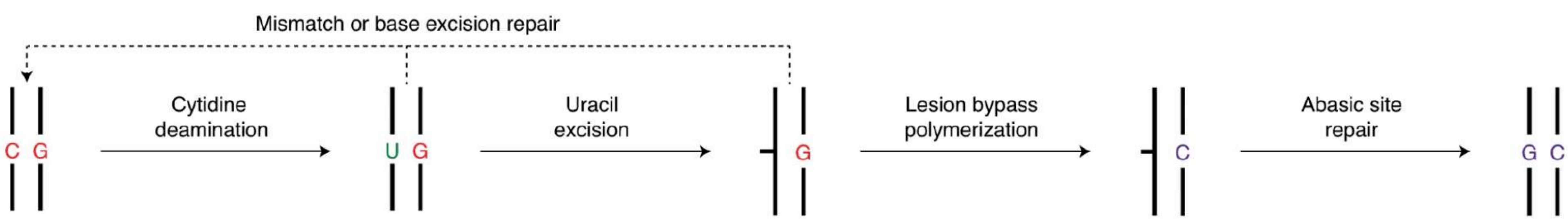
Base editing

CG base editors (CGBEs)





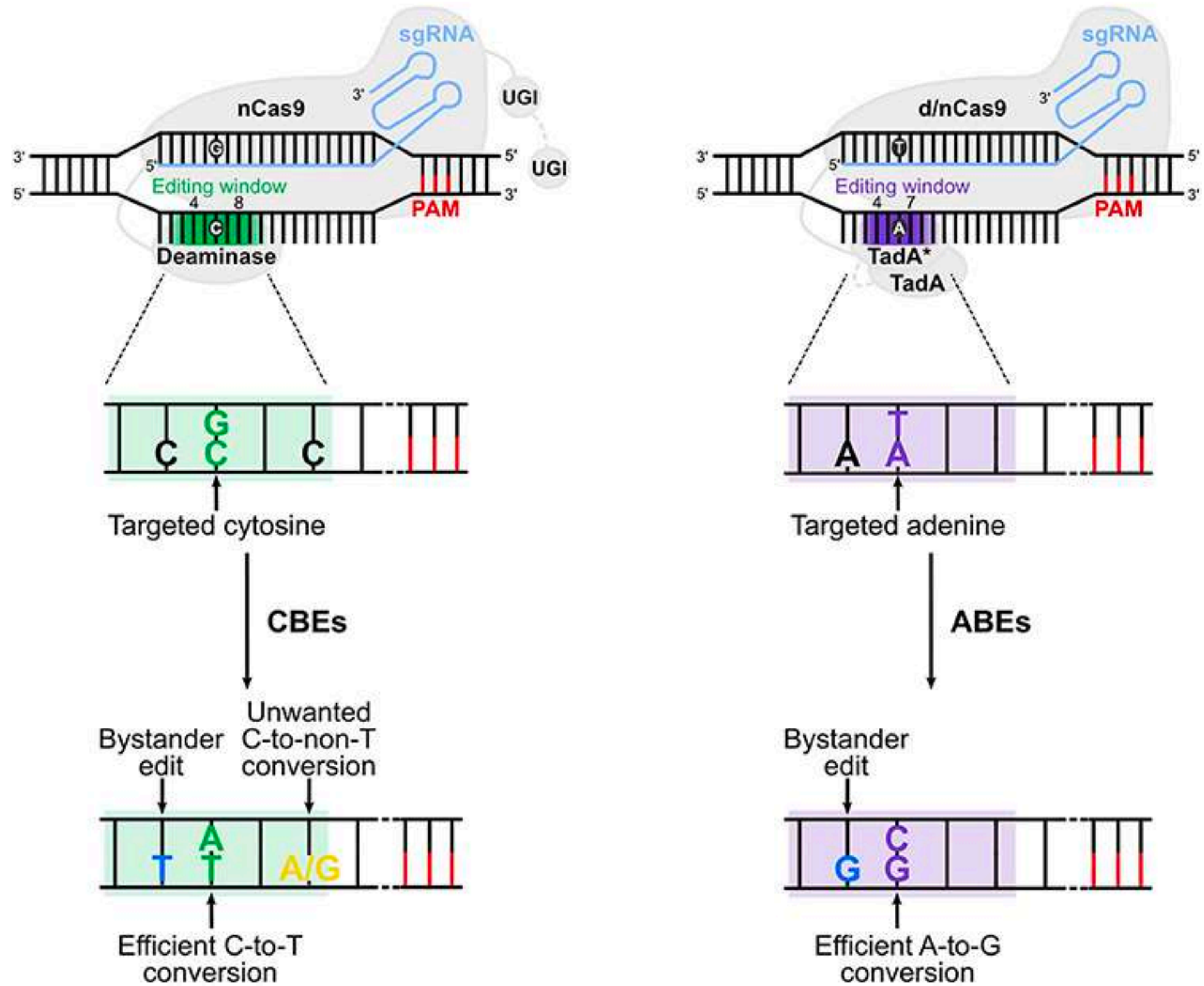
cytidine (C) uracil (~T)



Nat. Biotech., **2021,** *39*, 1414-1425.

Base editing

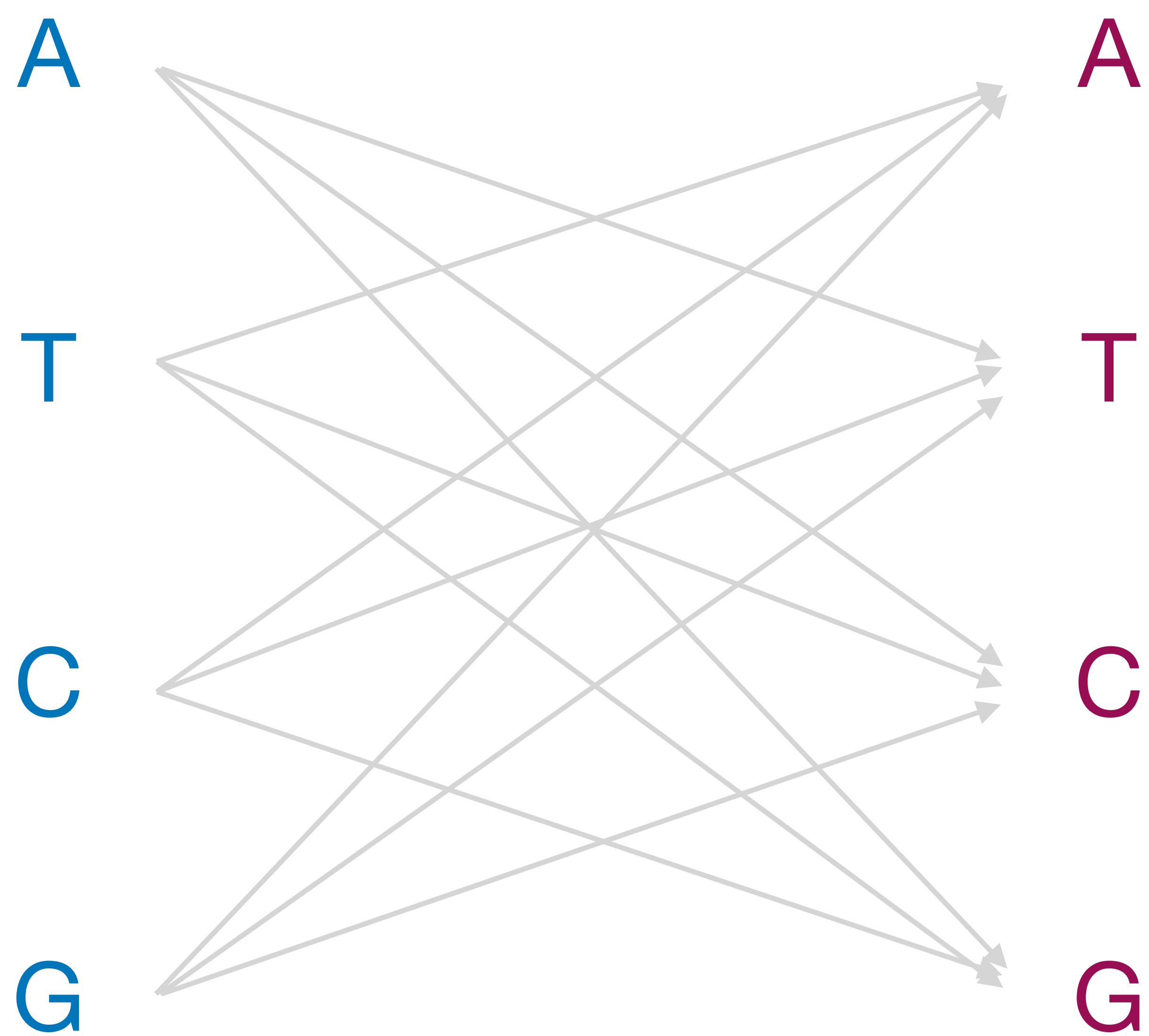
…isn't perfect



Nat. Rev. Drug. Disc., **2020,** *19*, 839-859.

Base editing

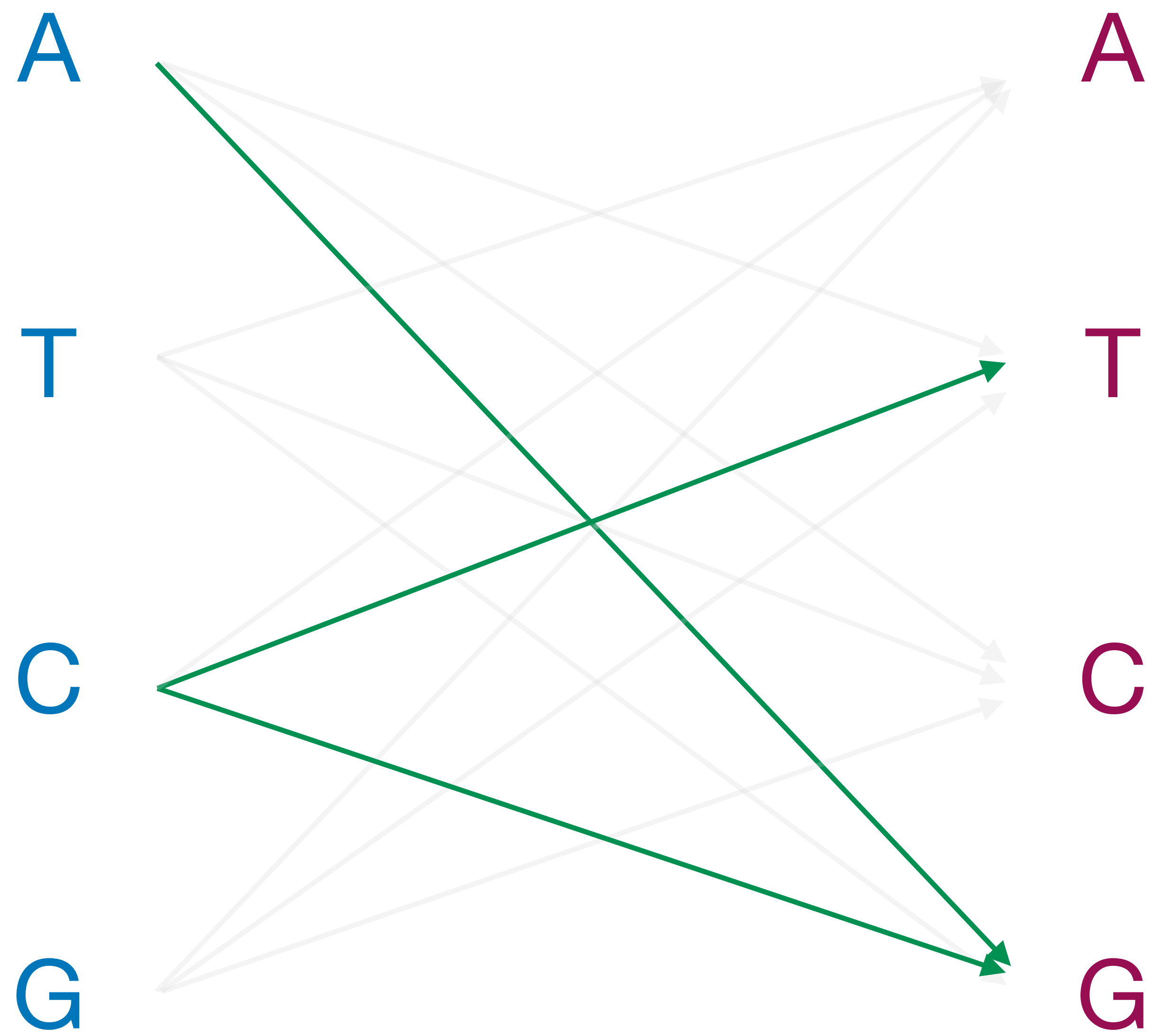
…is limited in scope



Nat. Rev. Drug. Disc., **2020,** *19*, 839-859.

Base editing

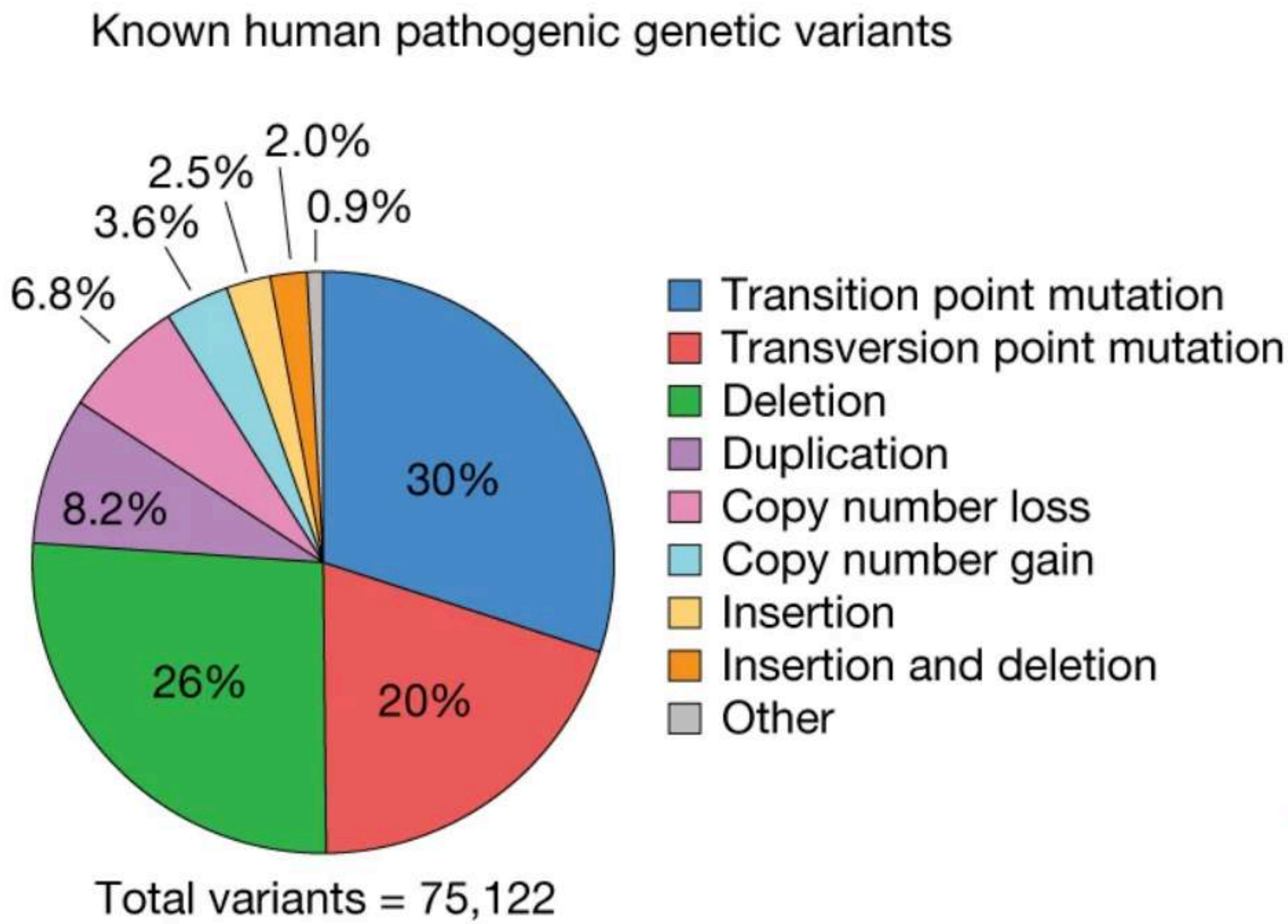
…is limited in scope



Nat. Rev. Drug. Disc., **2020,** *19*, 839-859.

Base editing

…is limited in scope

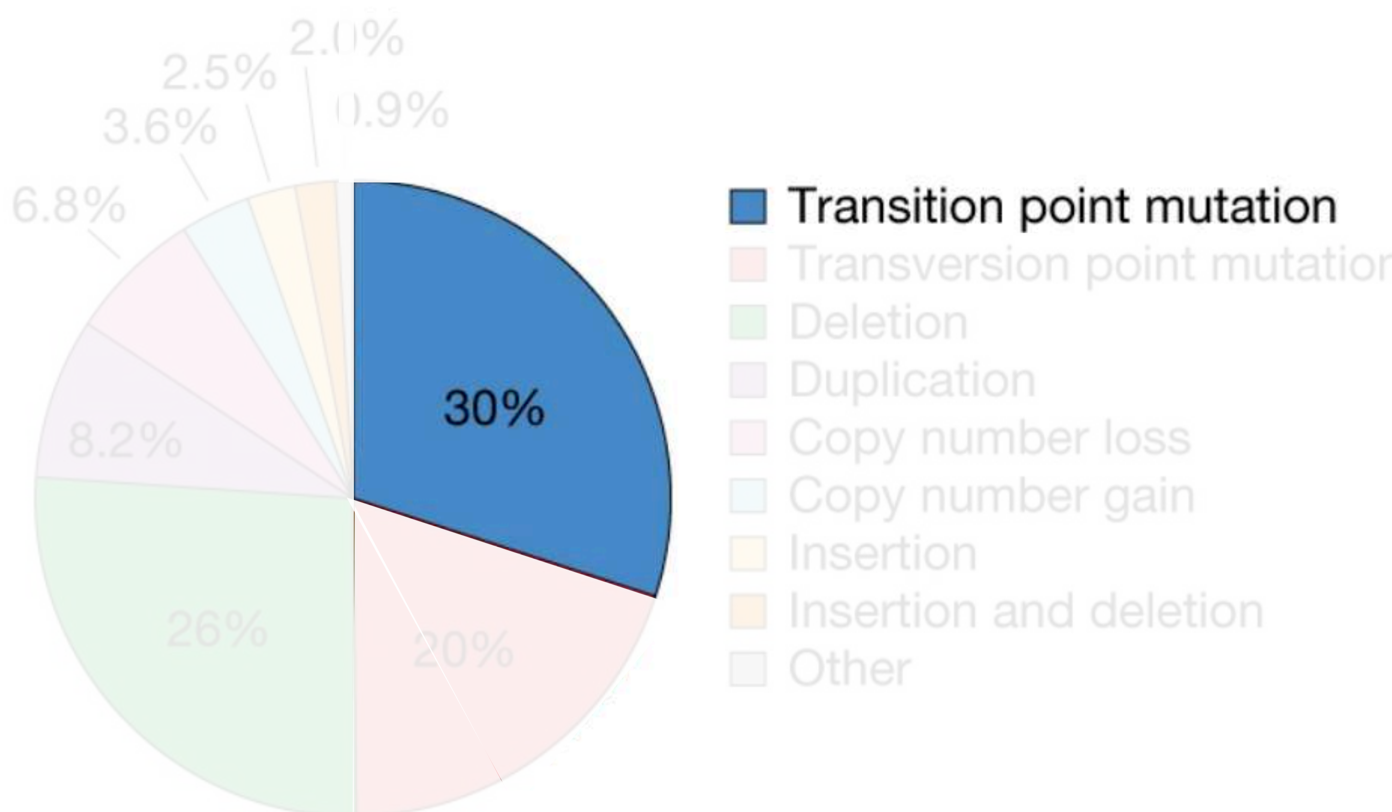


Nat. Rev. Drug. Disc., **2020,** *19*, 839-859.

Base editing

…is limited in scope

Known human pathogenic genetic variants

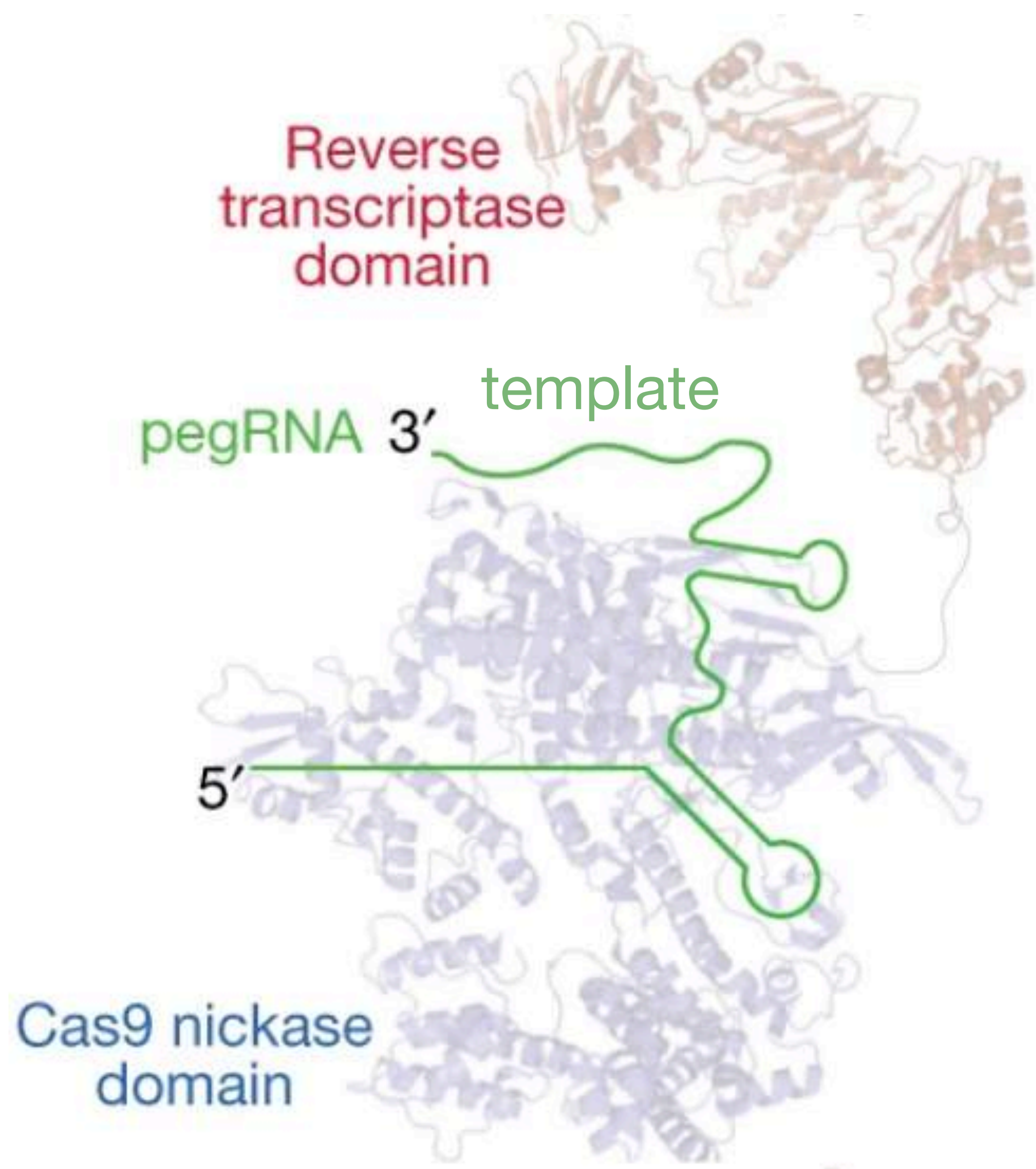


Total variants = 75,122

Nat. Rev. Drug. Disc., **2020,** *19*, 839-859.

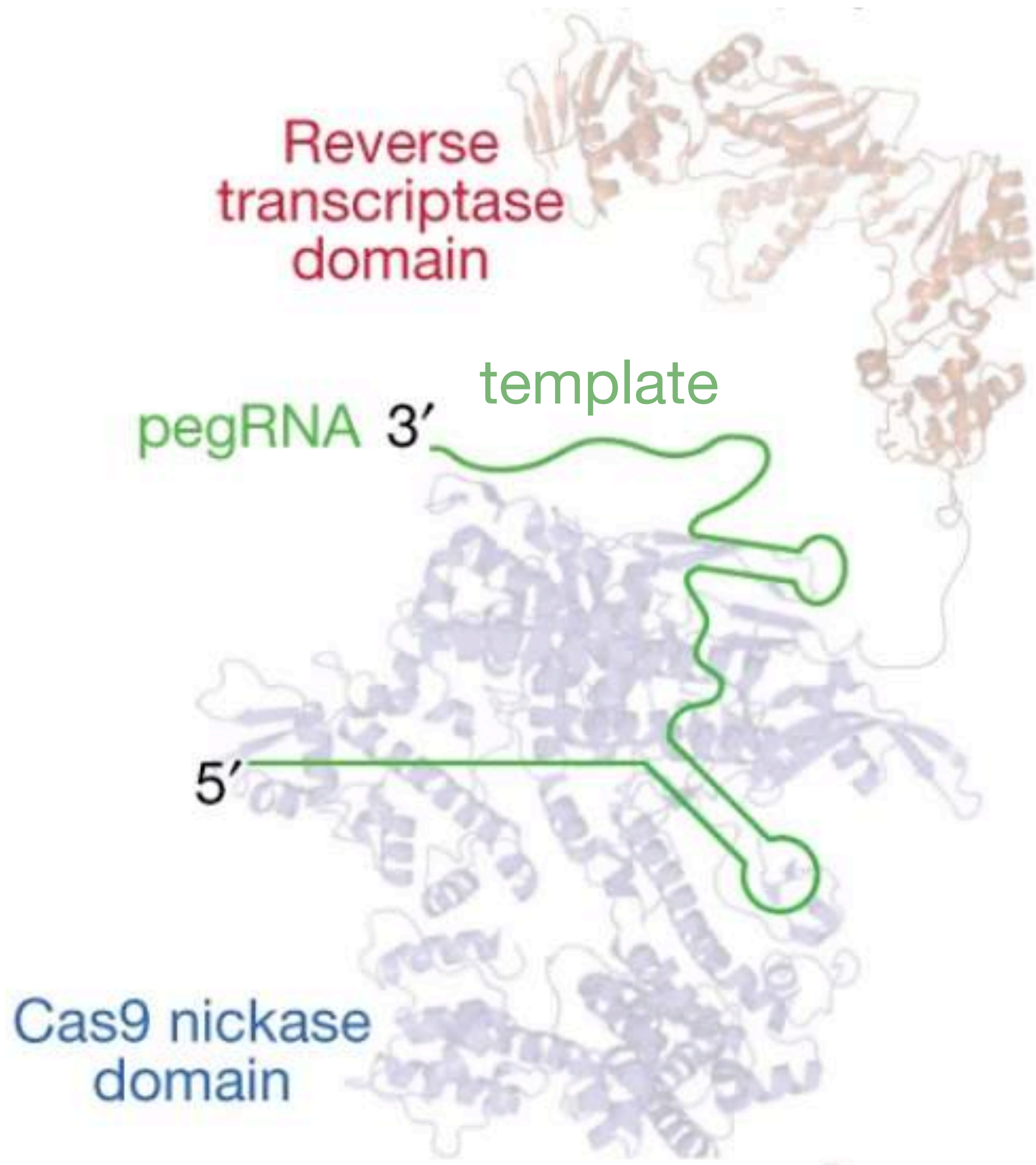
Prime editing

<u>**p**</u>rime <u>**e**</u>ditor <u>**g**</u>uide <u>**RNA**</u>



Nature, **2019,** *576*, 149-157.

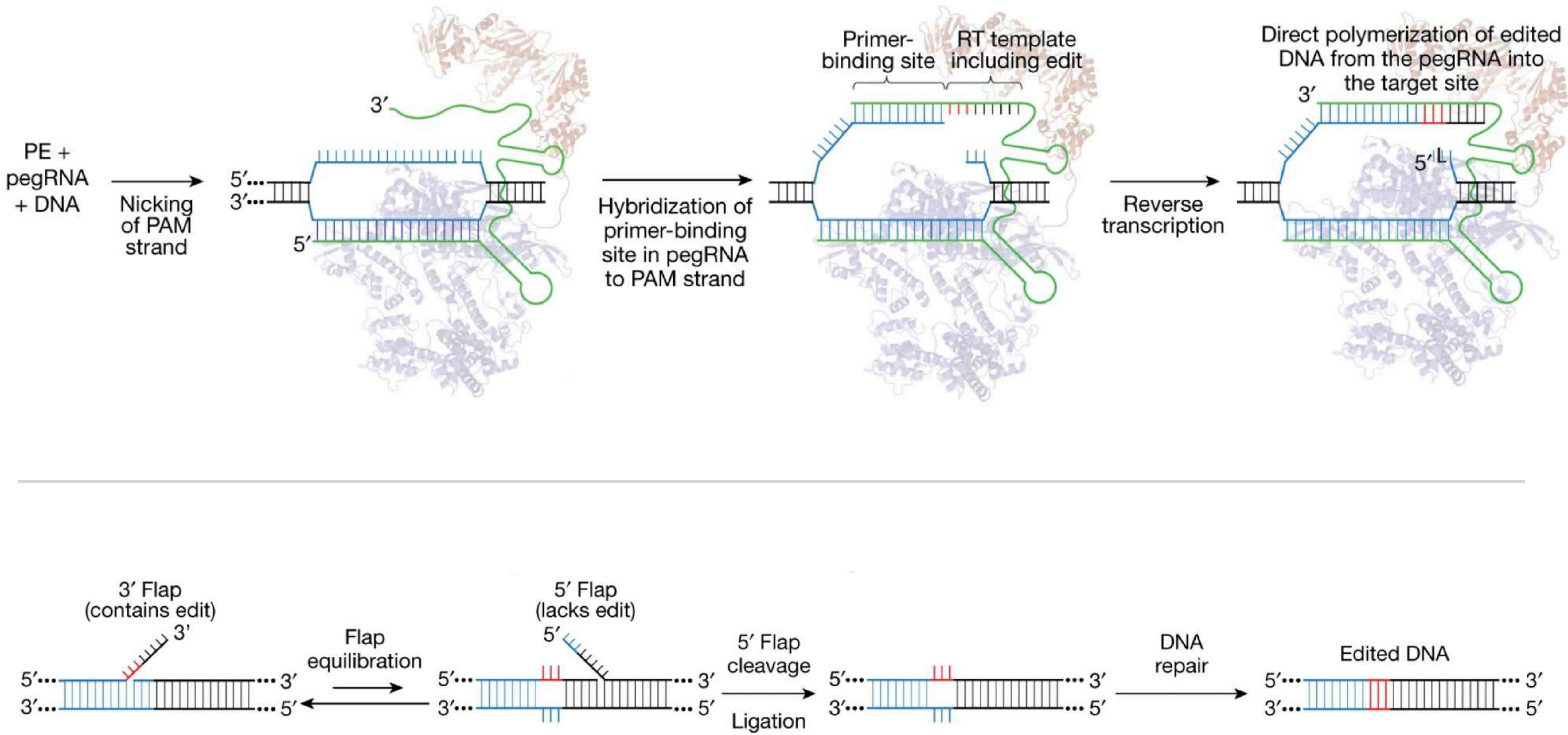
Prime editing



- recruits Cas9 to specific loci
- encodes the edit

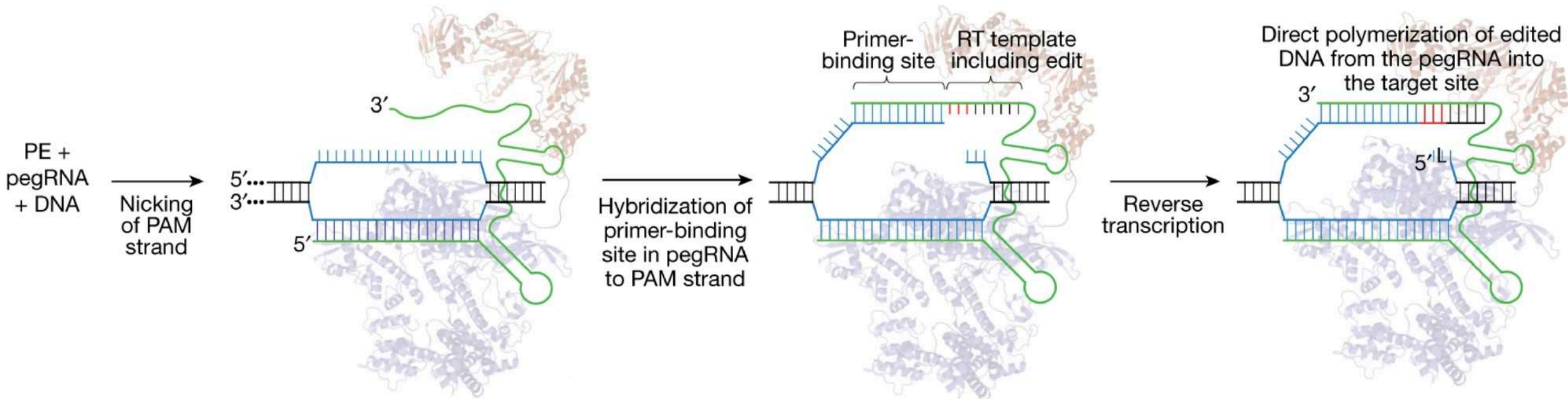
Nature, **2019,** *576*, 149-157.

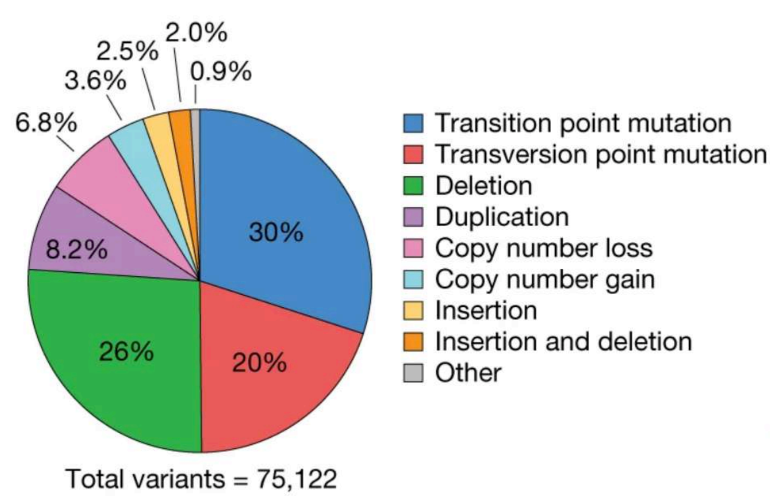
Prime editing



Nature, **2019,** *576*, 149-157.

Prime editing

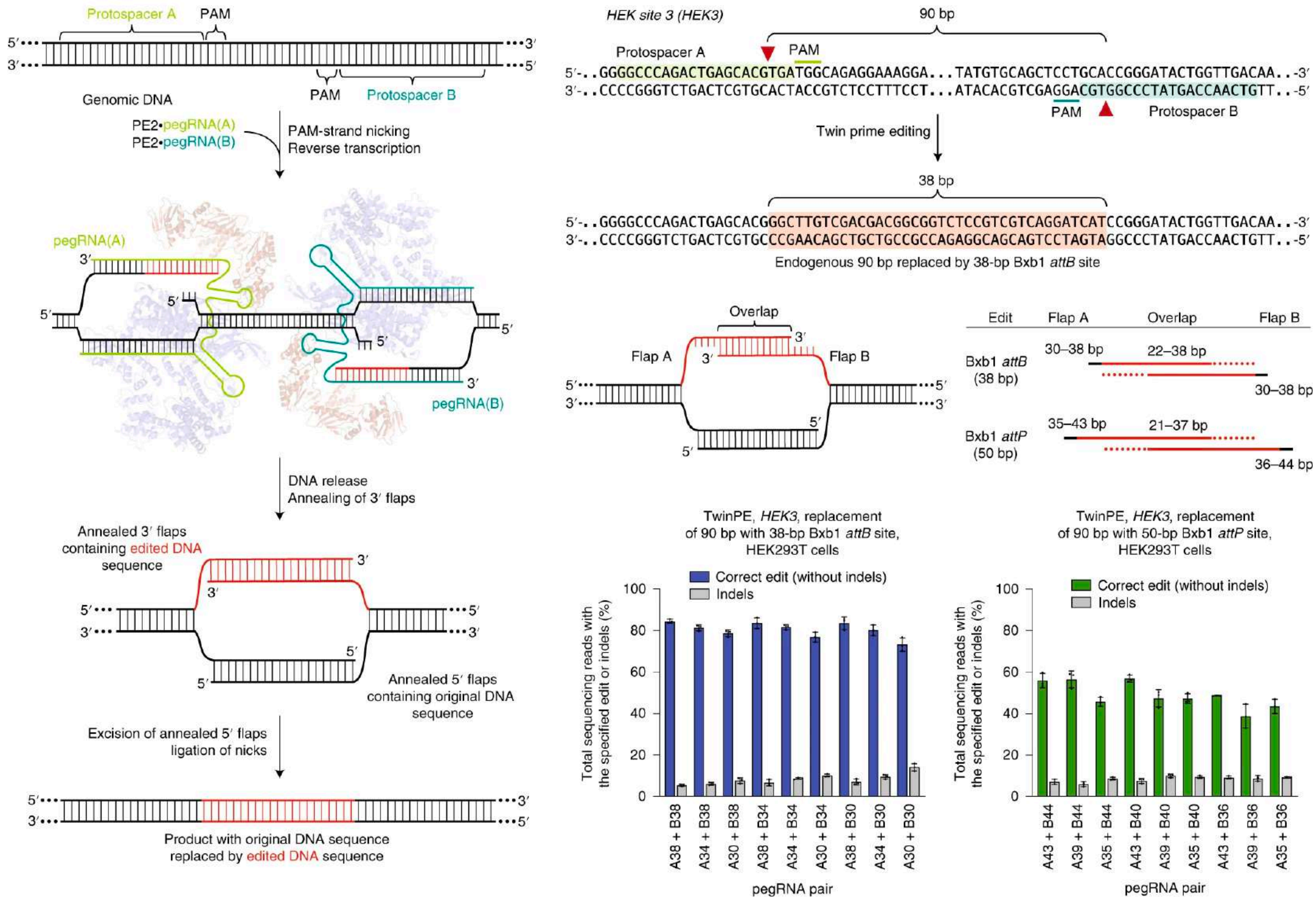




- all 4 transitions
- all 8 transversions
- insertions (1 - 50 bp)
- deletions (1 - 80 bp)
- combinations of the above

Nature, **2019,** *576*, 149-157.

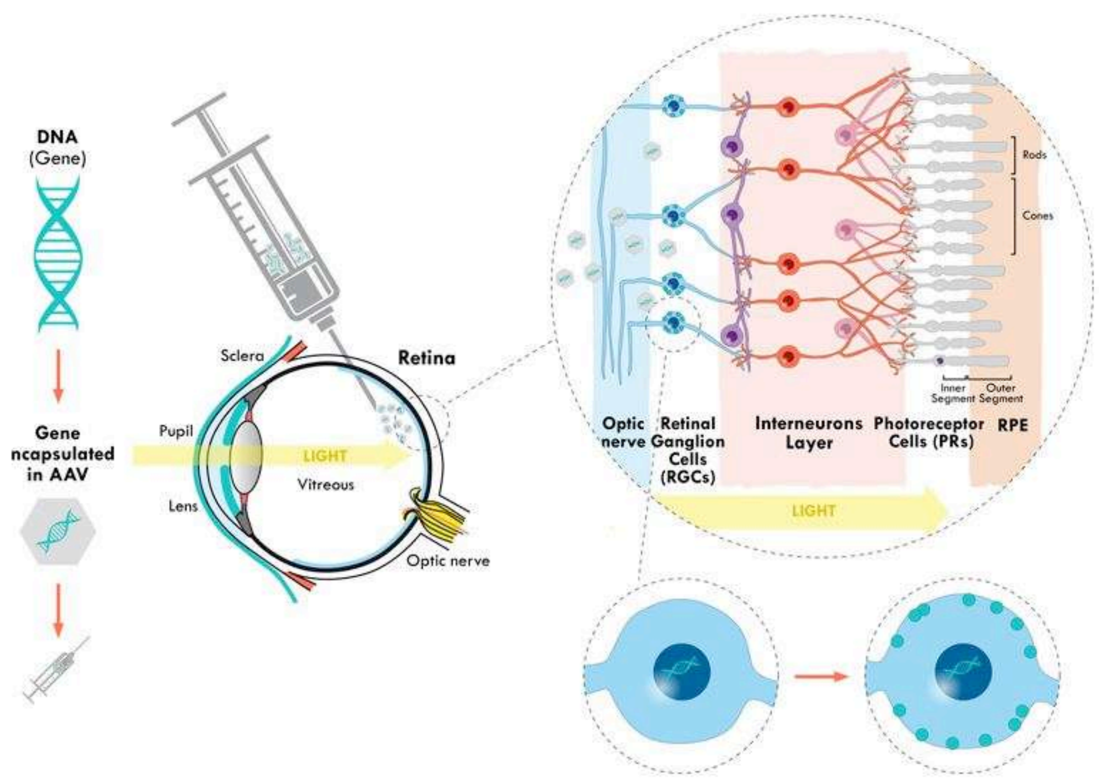
"Twin" Prime editing

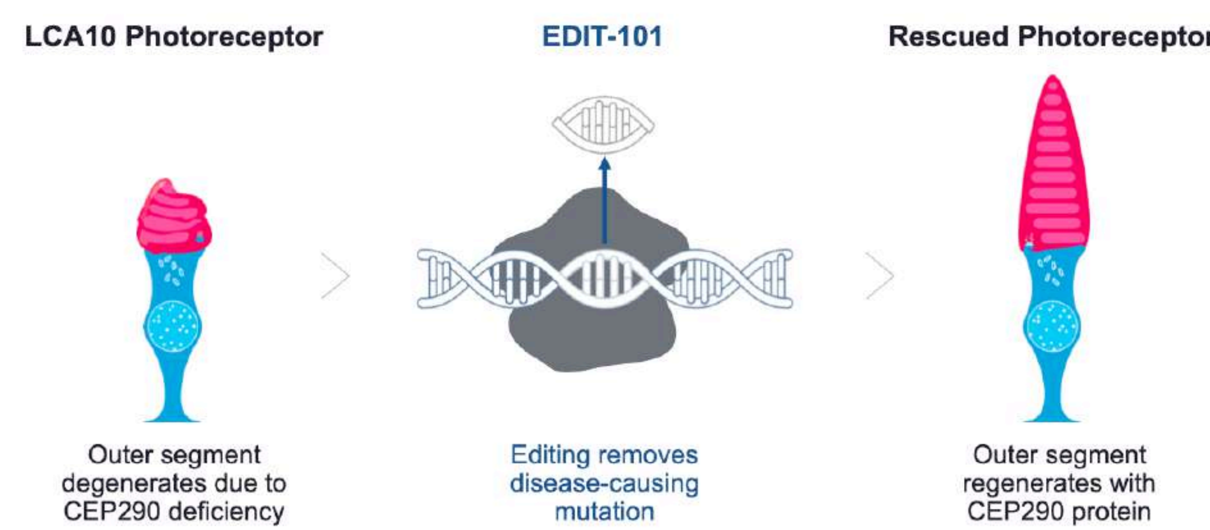


Nat. Biotech, **2022,** *40*, 731-740.

In vivo editing - current clinical applications

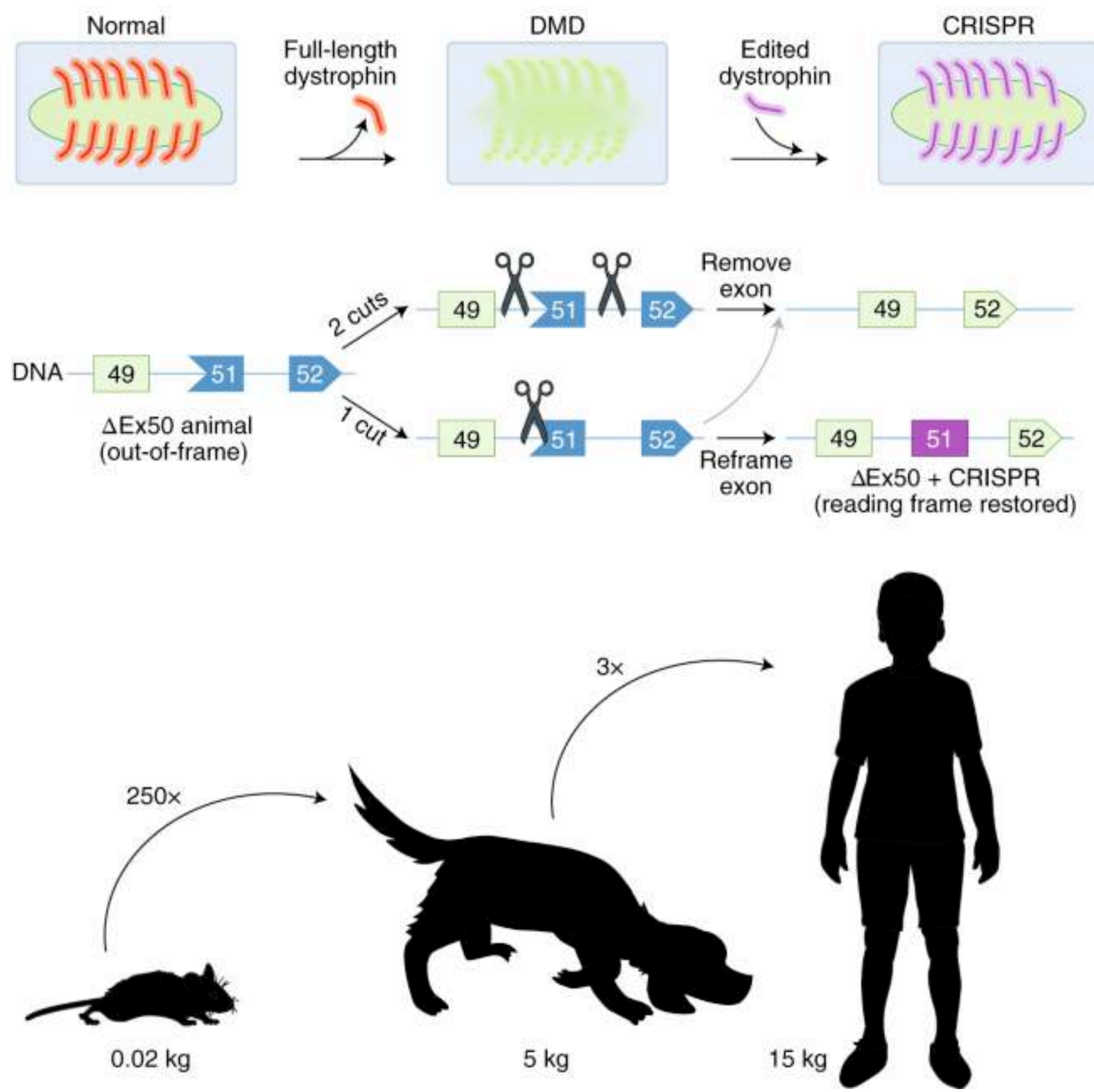
editas MEDICINE





In vivo editing - current clinical applications

Duchenne muscular dystrophy (DMD)



Science, **2018,** *362*, 86-91.

In vivo editing - current clinical applications

base editors

Table 1 | Recent examples of preclinical work with DNA base editors in disease models

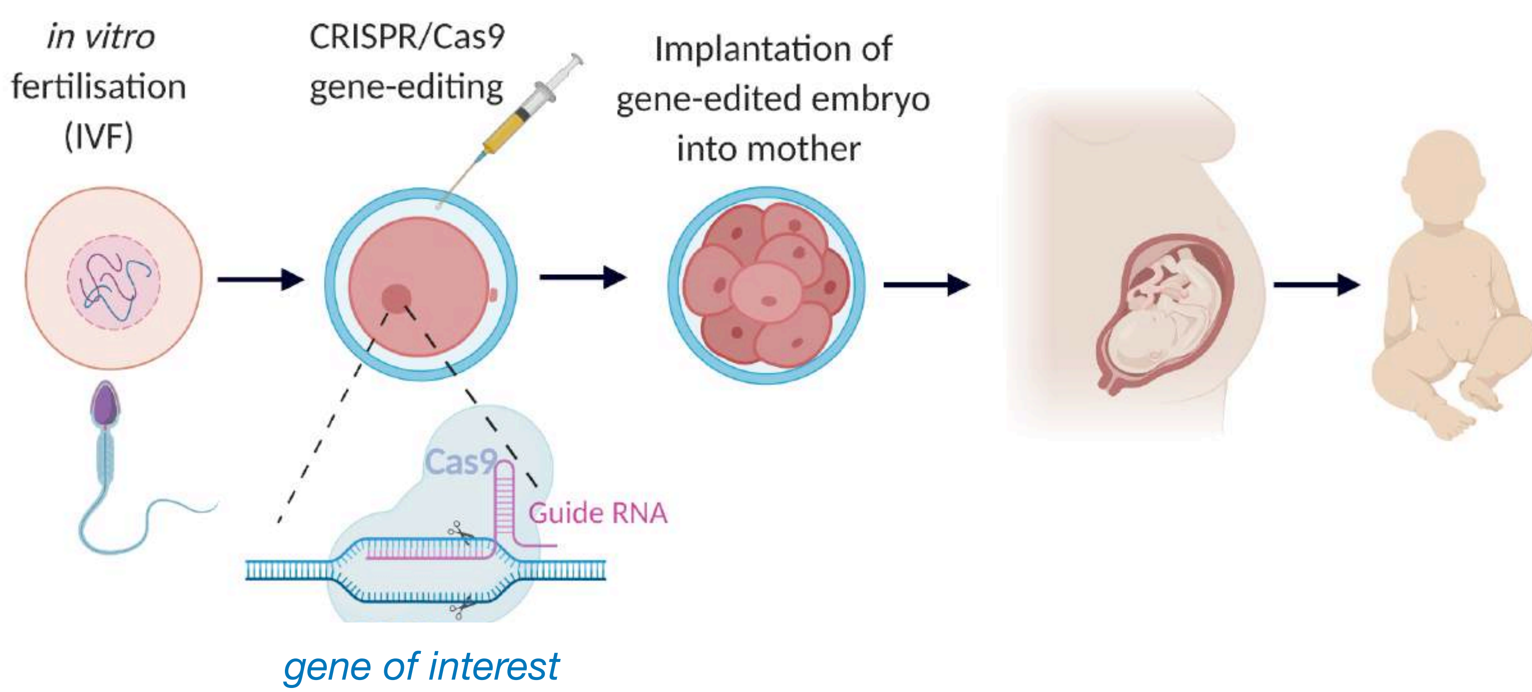
Disease targeted	**Gene targeted**	**Model**	**Reference**
HIV	*CCR5*, *CXCR4*	Human T cells and hematopoietic stem cells	Knipping, F. et al. *Mol. Ther.* https://doi.org/10.1016/j.ymthe.2021.10.026 (2021).
Leber congenital amaurosis	*RPE65*	In vivo mouse model	Choi, E. H. *Nat. Commun.* https://doi.org/10.1038/s41467-022-29490-3 (2022).
Adrenoleukodystrophy	*ABCD1*	In vivo mouse model	Hong, S.-A. *Mol. Ther.* https://doi.org/10.1016/j.ymthe.2021.05.022 (2021).
Hereditary tyrosinemia type 1	*FAH*	Mouse hepatocytes	Kim, Y. et al. *Cell Stem Cell* https://doi.org/10.1016/j.stem.2021.04.010 (2021).
Hurler syndrome	*IDUA*	In vivo mouse model	Bose, S. K. et al. *Nat. Commun.* https://doi.org/10.1038/s41467-021-24443-8 (2021).
Sickle cell disease	*HBB*	In vivo mouse model	Newby, G. A. et al. https://doi.org/10.1038/s41586-021-03609-w (2021).
Duchenne muscular dystrophy	*DMD*	In vivo mouse model	Xu, L. et al. *Nat. Commun.* https://doi.org/10.1038/s41467-021-23996-y (2021).
Atherosclerotic cardiovascular disease	*PCSK9*	In vivo non-human primate model	Musunuru, K. et al. *Nature* https://doi.org/10.1038/s41586-021-03534-y (2021).
Hutchinson–Gilford progeria	*LMNA*	In vivo mouse model	Koblan, L. W. et al. *Nature* https://doi.org/10.1038/s41586-020-03086-7 (2021).

Nat. Rev. Drug. Disc., **2020,** *19*, 839-859.

Editing embryos

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

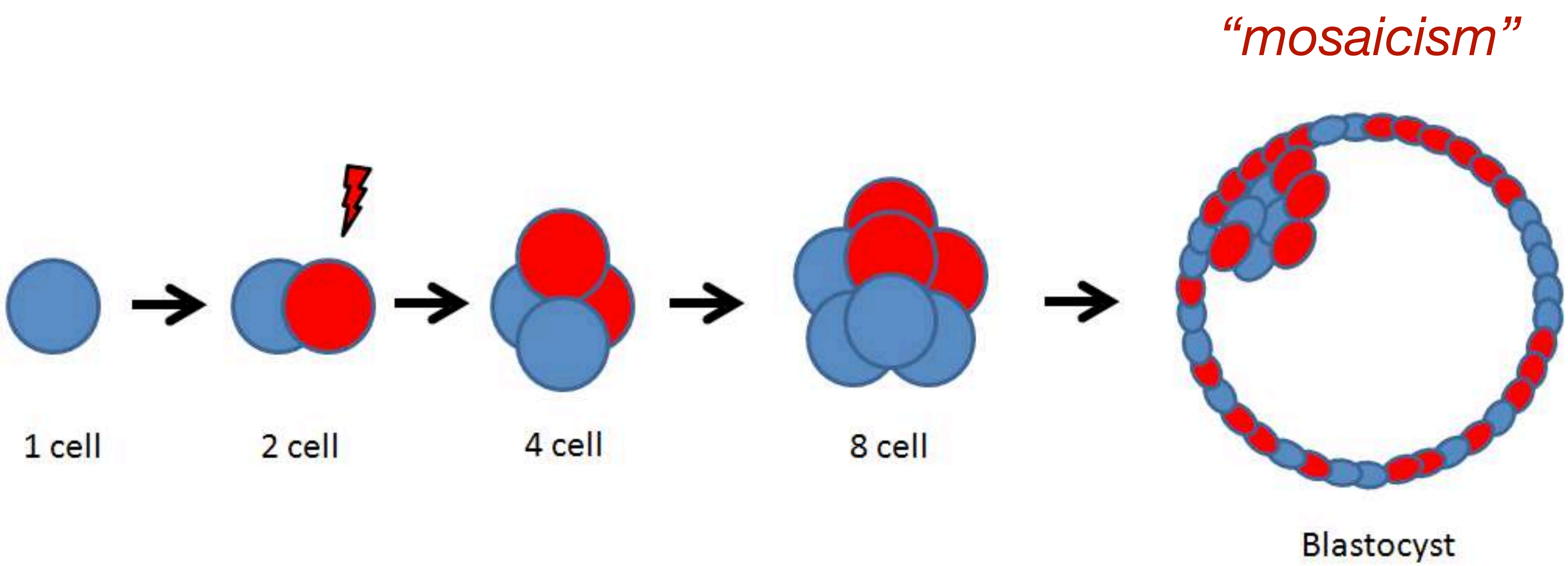


Nature, **2017,** *548*, 413-419.

first 30 min - 15 h

… and every 15-24 h hence

Editing embryos



Nature, **2017,** *548*, 413-419.

Editing embryos

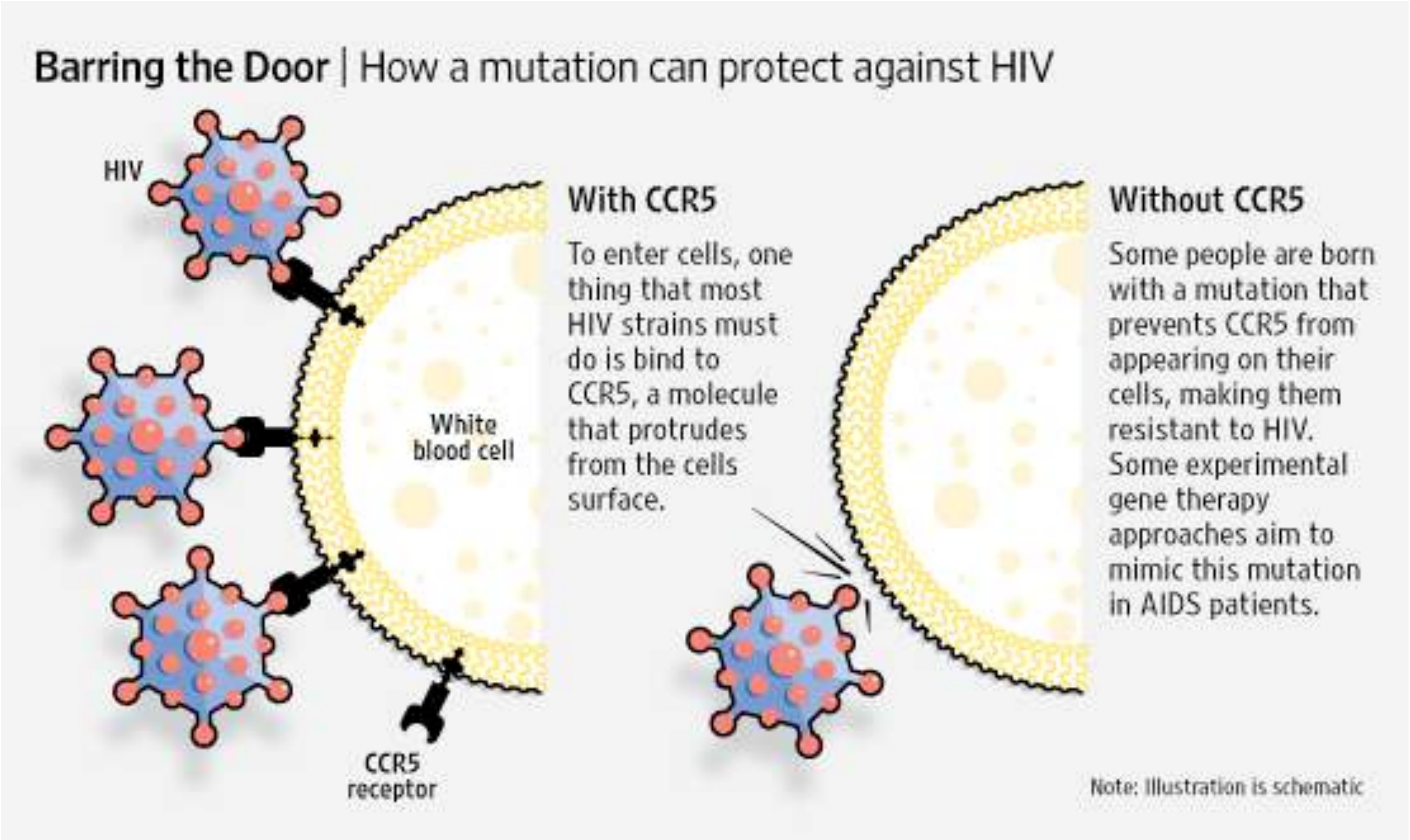



Nature, **2017,** *548*, 413-419.

Lulu and Nana

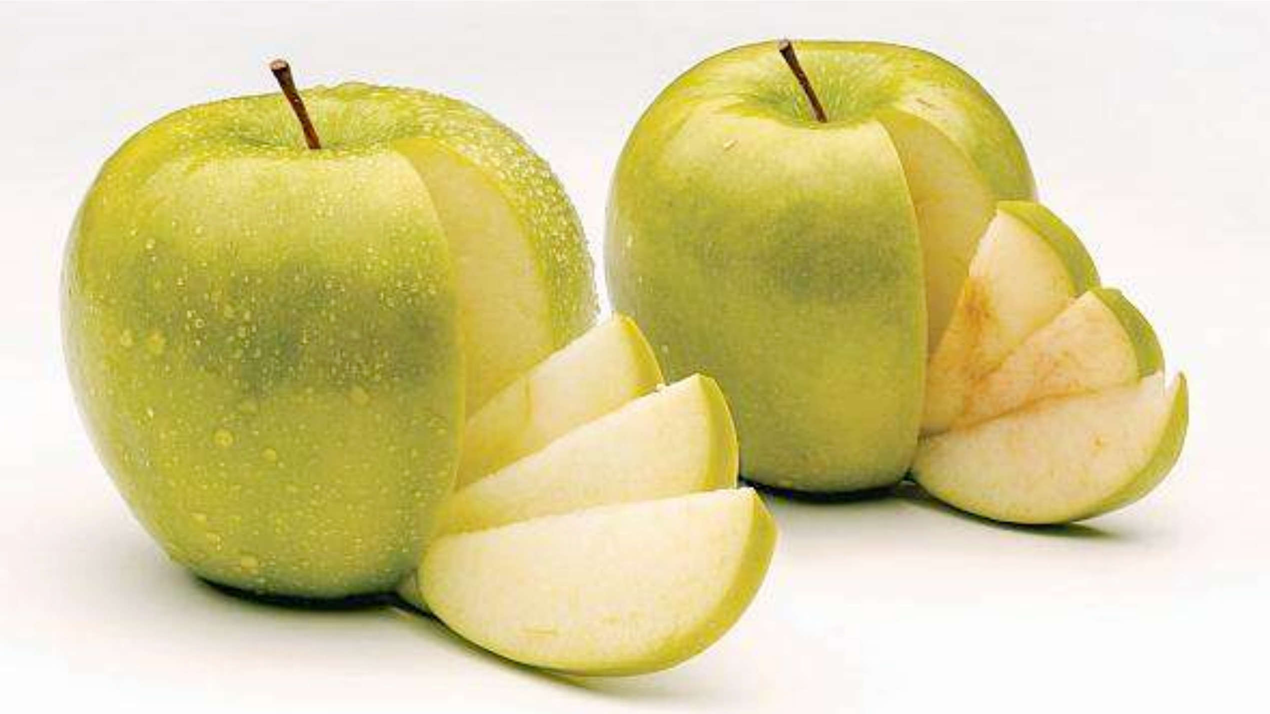
He Jiankui

SUSTech, Shenzhen



- 2017-2018: attempt CCR5 deletion in embryos from HIV-positive couples
- homozygous deletion of CCR5 does confer resistance to HIV
- heterozygote mosaics were obtained, but proceeded anyway (shorter life expectancy)
- Lulu and Nana born Oct 2018 as first engineered humans
- International outrage, Jiankui sent to prison

The agricultural revolution



Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

The agricultural revolution

CARLA GOTTGENS/BLOOMBERG/GETTY

Workers inspect a banana harvest at a farm in Australia.

GENETICS

CRISPR could save bananas from fungus

Researchers are using the gene-editing tool to boost the fruit's defences against a deadly pathogen.

Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

Broad-spectrum resistance to bacterial blight in rice using genome editing

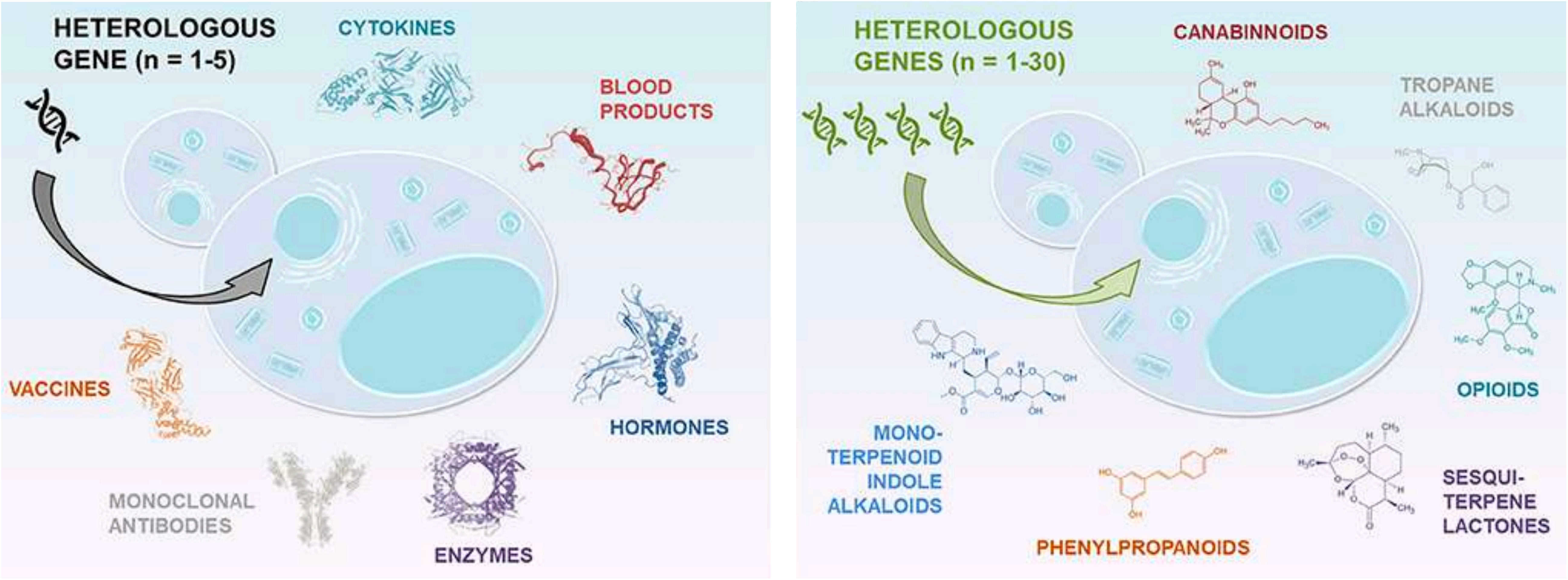
Ricardo Oliva[1,12*], Chonghui Ji[2,12], Genelou Atienza-Grande[1,10,12], José C. Huguet-Tapia[3,12], Alvaro Perez-Quintero[4,11,12], Ting Li[5], Joon-Seob Eom[6], Chenhao Li[2], Hanna Nguyen[1], Bo Liu[2], Florence Auguy[4], Coline Sciallano[4], Van T. Luu[6], Gerbert S. Dossa[7], Sébastien Cunnac[4], Sarah M. Schmidt[6], Inez H. Slamet-Loedin[1], Casiana Vera Cruz[1], Boris Szurek[4], Wolf B. Frommer[6,8*], Frank F. White[3] and Bing Yang[2,9*]

Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9

Oliver Xiaoou Dong[1,2,3], Shu Yu[4], Rashmi Jain[1], Nan Zhang[1], Phat Q. Duong[1], Corinne Butler[1], Yan Li[1], Anna Lipzen[5], Joel A. Martin[5], Kerrie W. Barry[5], Jeremy Schmutz[5], Li Tian[4] & Pamela C. Ronald[1,2,3]✉

Nat. Rev. Mol. Cell. Biol., **2020**, *21*, 661-677.

Yeast and bacterial bioproduction platforms



Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

Yeast and bacterial bioproduction platforms

Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

Transgenic animals

Argiope catenulata

Capra hircus

Proc. Natl. Acad. Sci., **2018,** *115*(35), 8757-8762.

Transgenic animals

George Church

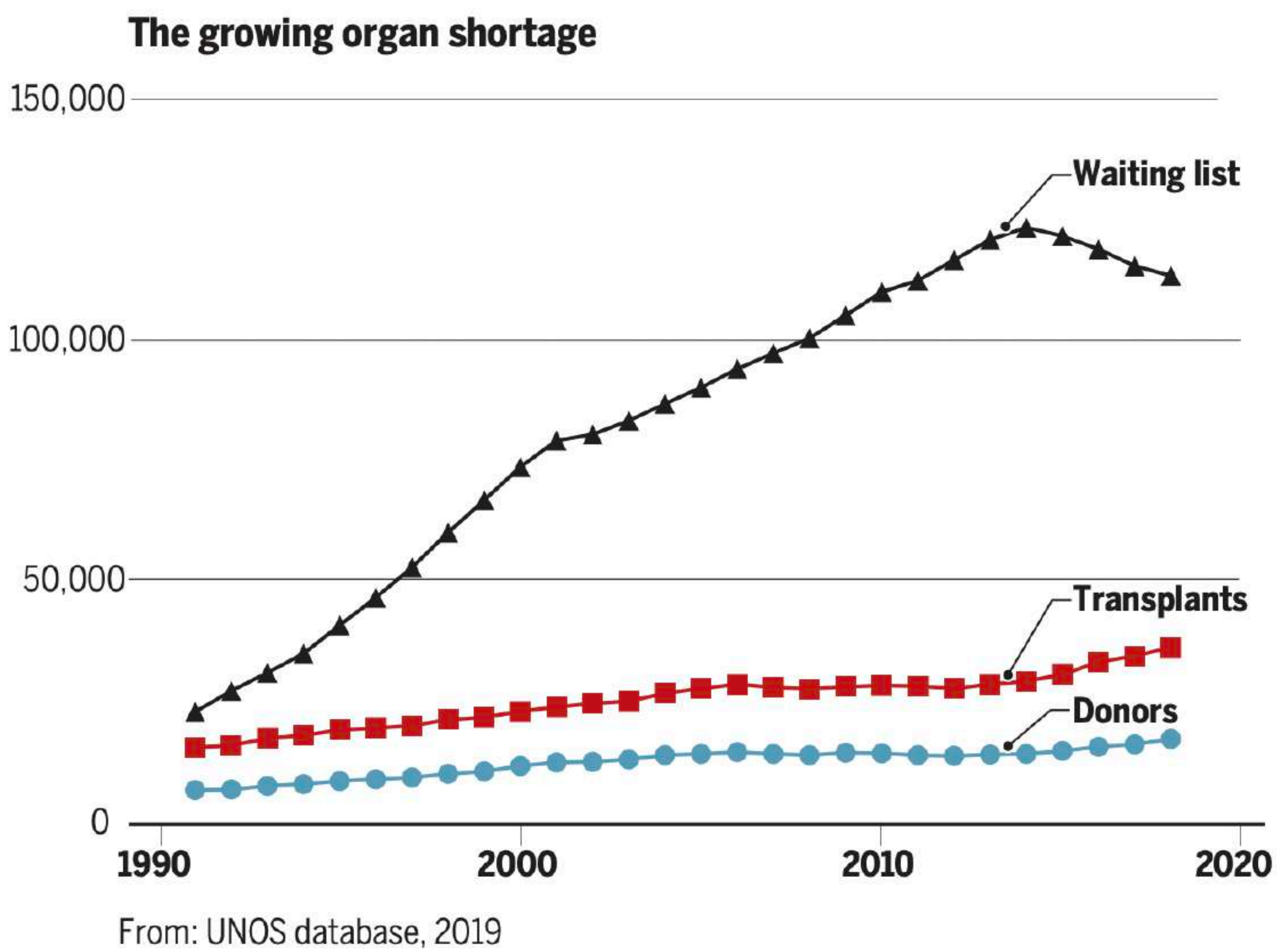
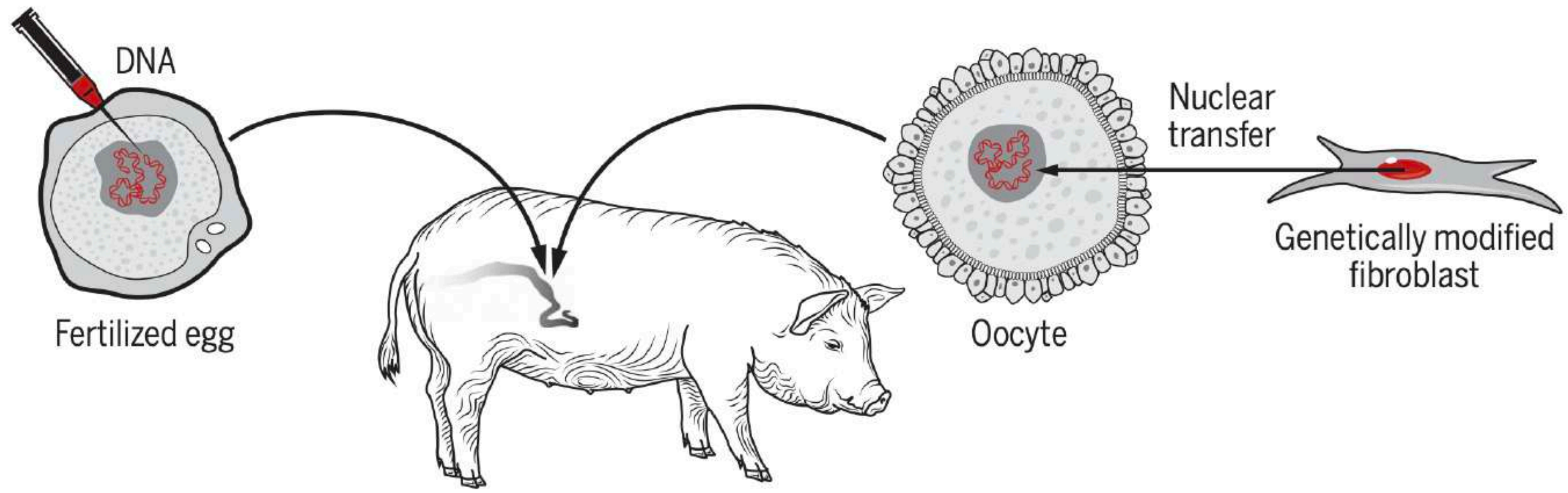


Fig. 1. The growing allogeneic organ supply/demand imbalance has resulted in an expanding transplant waiting list.

Sci. Immunol., **2019,** *4*(41).

Transgenic animals

George Church



Transgenics

Complement inhibition
- hDAF
- hCD46
- hCD59

Coagulation inhibition
- hCD39
- Human thrombomodulin

Anti-inflammatory genes
- HO-1
- A20

Immunosuppressive molecules
- Anti-CD2
- CTLA4Ig
- hCD47

PERV siRNA

MHC genes
- Class I (NK inhibition)

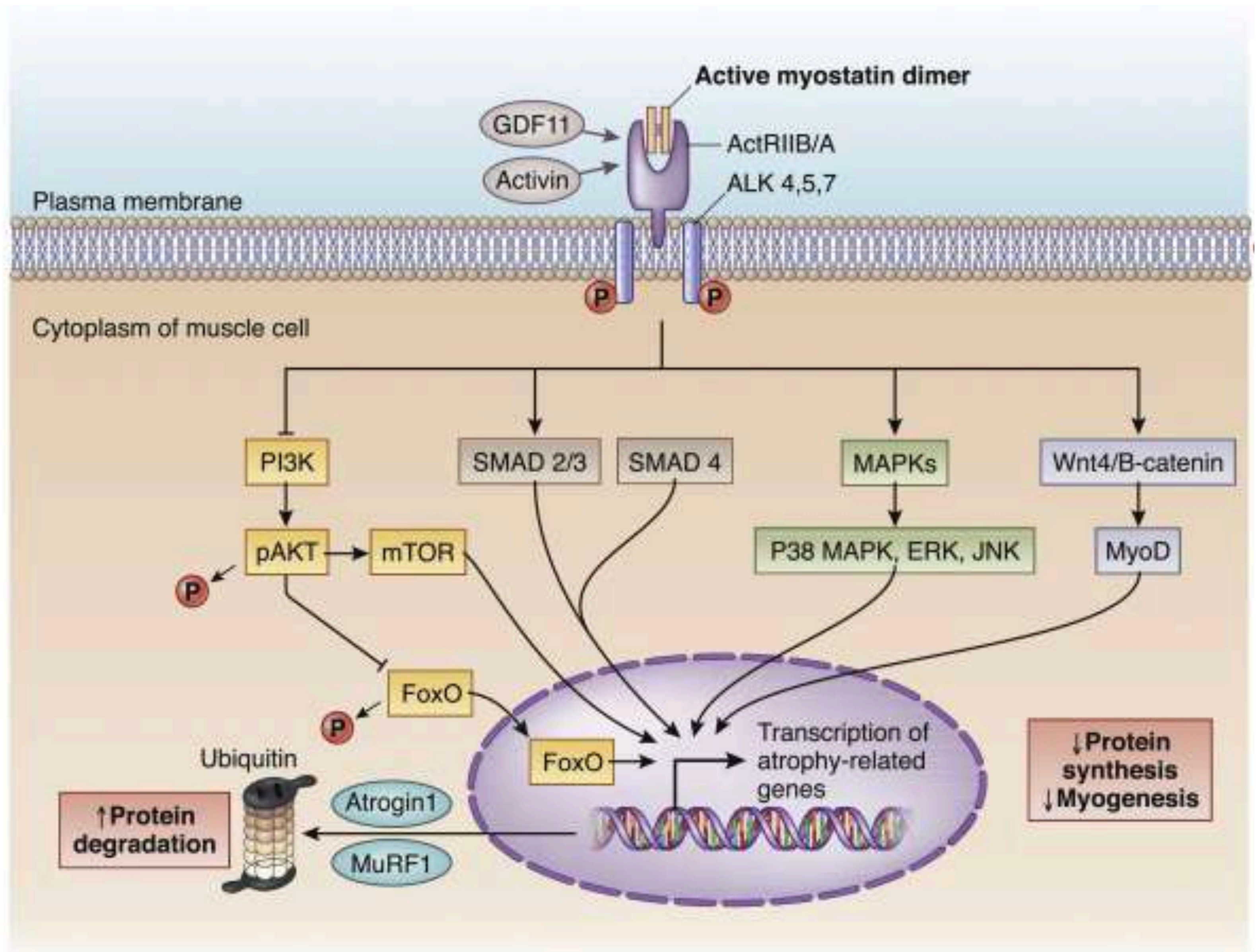
Knockouts

- α1,3-galactosyltransferase
- CMAH
- B4GalNT2
- vWF
- PERVs

Fig. 3. Genetic modifications that have been made in pigs to facilitate pig-to-human organ transplantation.

Sci. Immunol., **2019,** *4*(41).

Myostatin inhibits muscle growth, blocking it can grow muscle

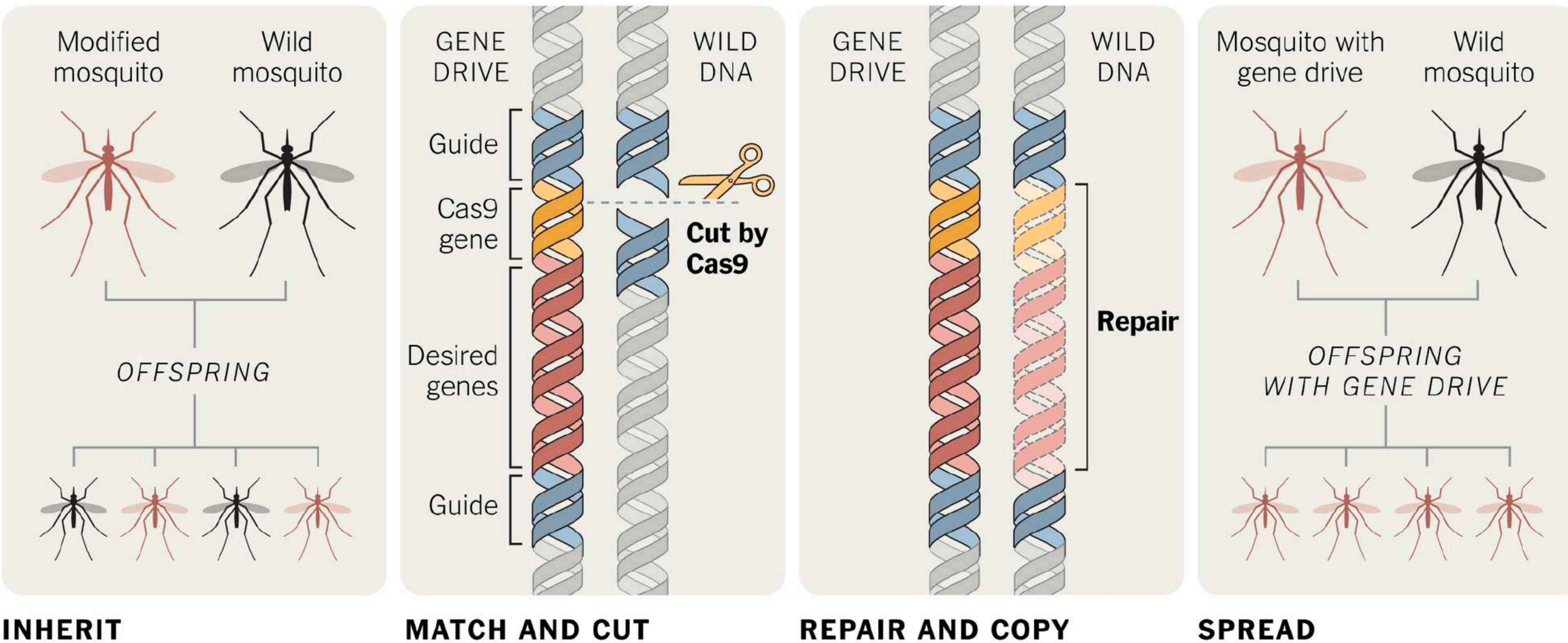


Nat. Comm., **2021,** *12,* 330.

Myostatin inhibits muscle growth, blocking it can grow muscle

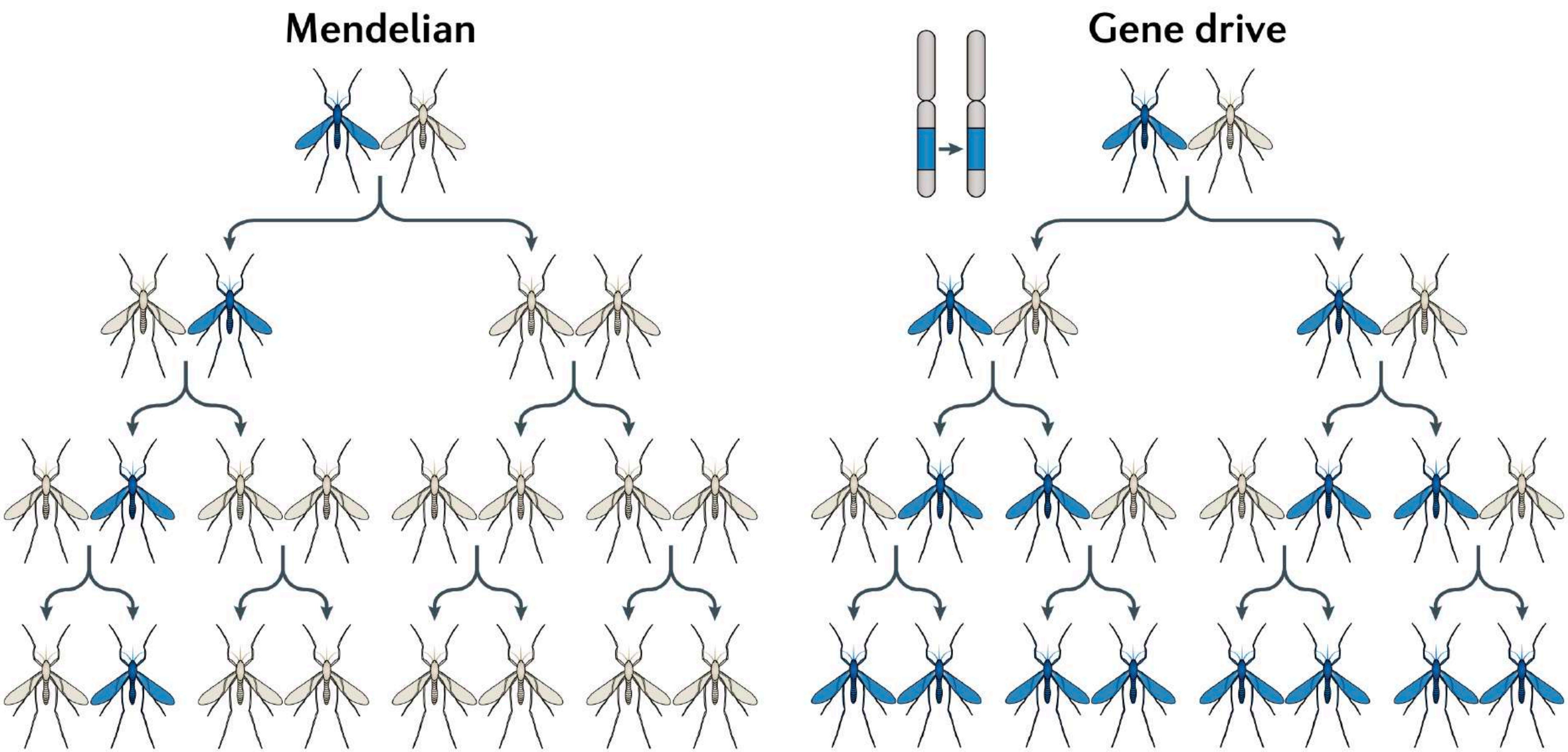
Nat. Comm., **2021,** *12,* 330.

Gene drives



Nat. Rev. Genetics, **2022,** *23,* 5-22.

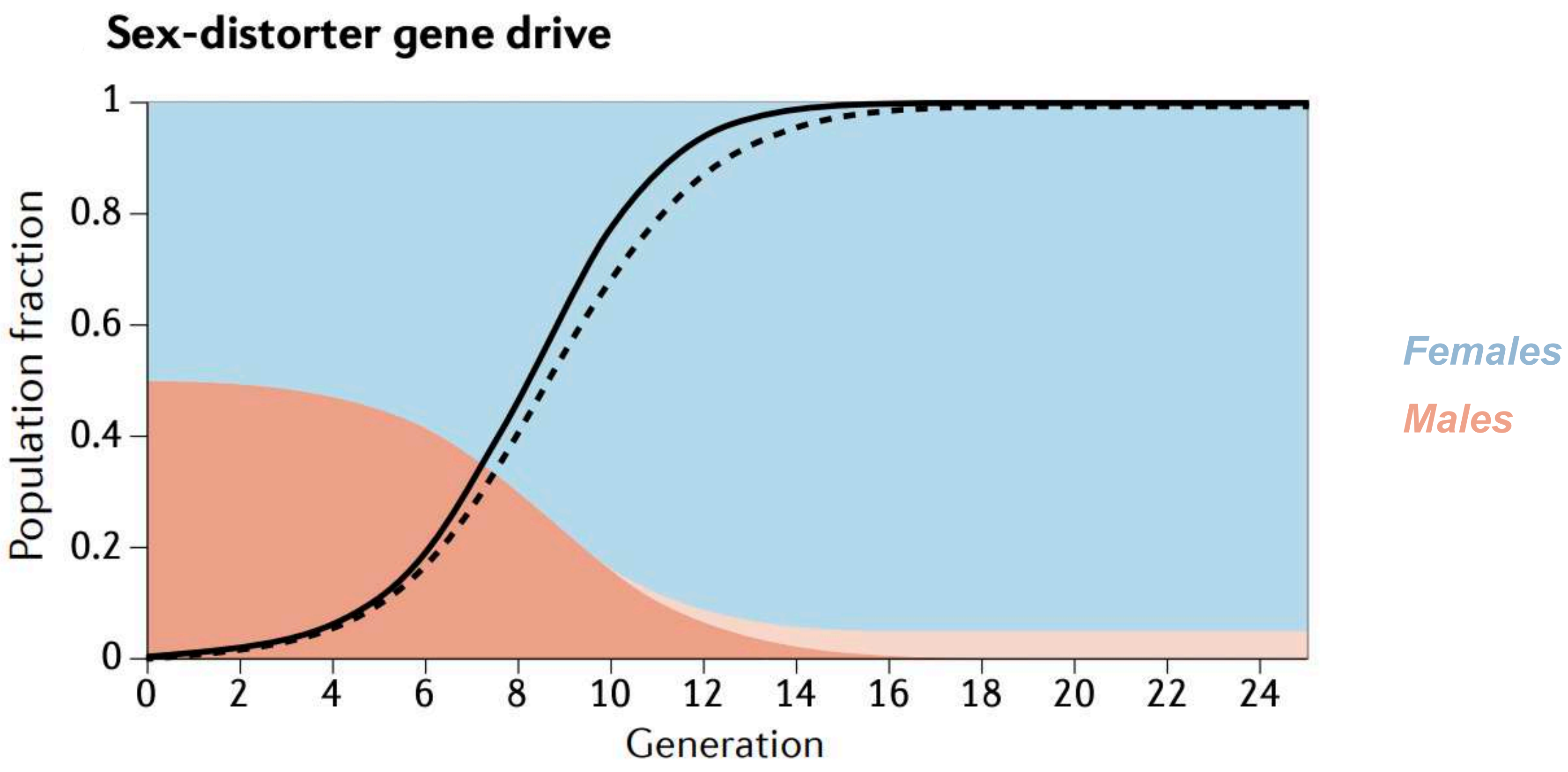
Gene drives



Nat. Rev. Genetics, **2022,** *23,* 5-22.

Gene drives

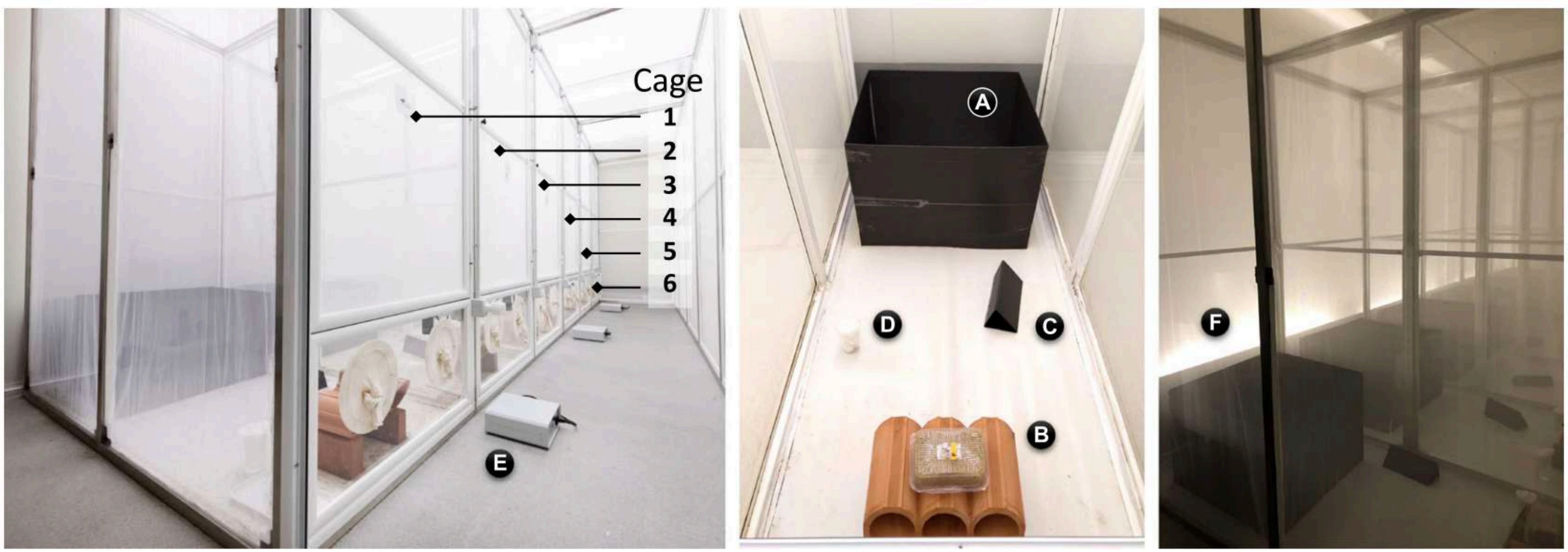
Anopheles gambiae



Nat. Rev. Genetics, **2022**, *23*, 5-22.

Gene-drive suppression of mosquito populations in large cages as a bridge between lab and field

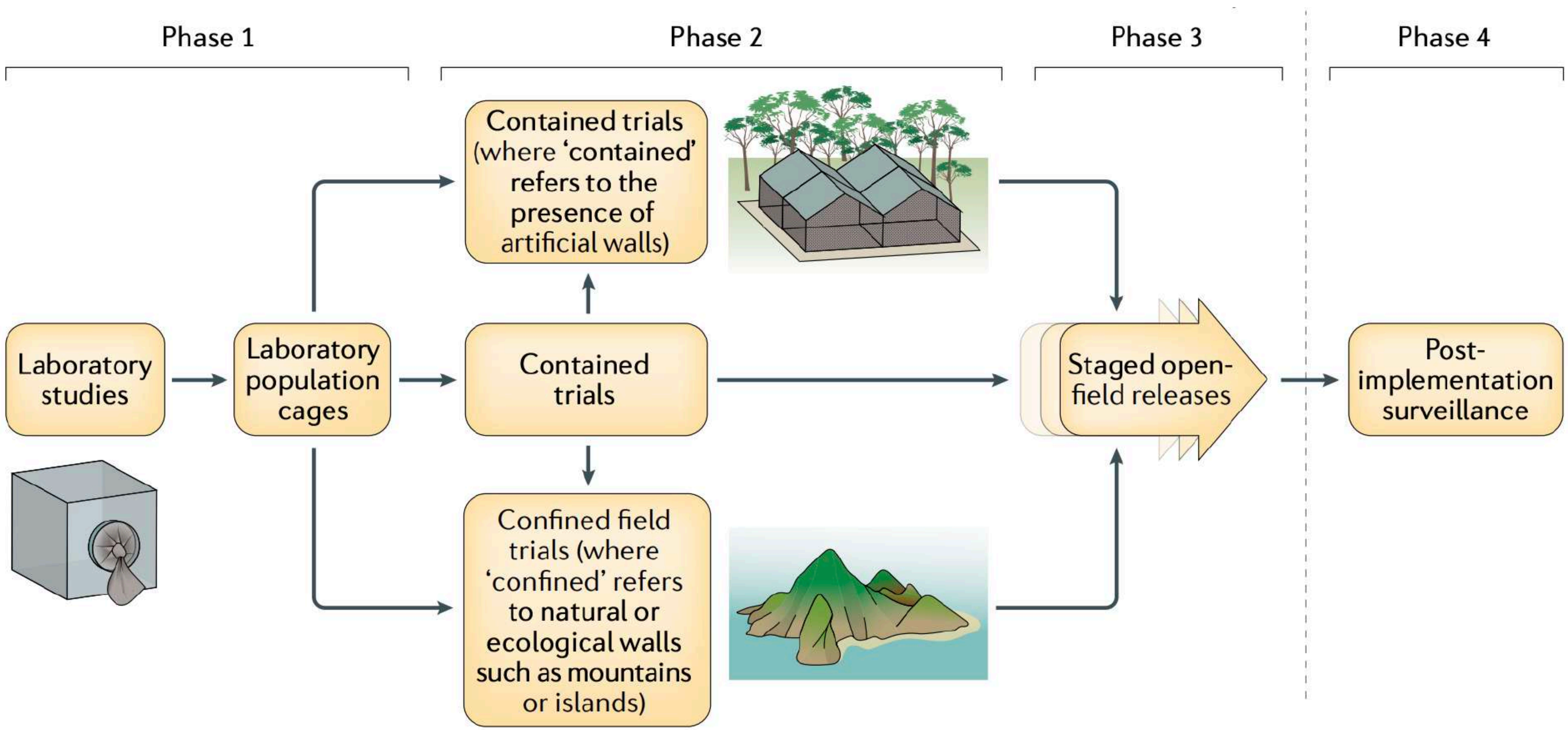
Andrew Hammond[1,2,9], Paola Pollegioni[3,4,9], Tania Persampieri[3,9], Ace North[5], Roxana Minuz[3], Alessandro Trusso[3], Alessandro Bucci[3], Kyros Kyrou[1], Ioanna Morianou[1], Alekos Simoni[1,3], Tony Nolan[1,6,10]✉, Ruth Müller[3,7,8,10]✉ & Andrea Crisanti[1,10]✉



(A) Swarming arena. (B) Wet resting site (bricks). (C) Dry resting site. (D) Glucose feeder. (E) Hemotek blood feeding system. (F) Sunset simulation

Nat. Rev. Genetics, **2022,** *23,* 5-22.

Gene drives



Nat. Rev. Genetics, **2022,** *23,* 5-22.

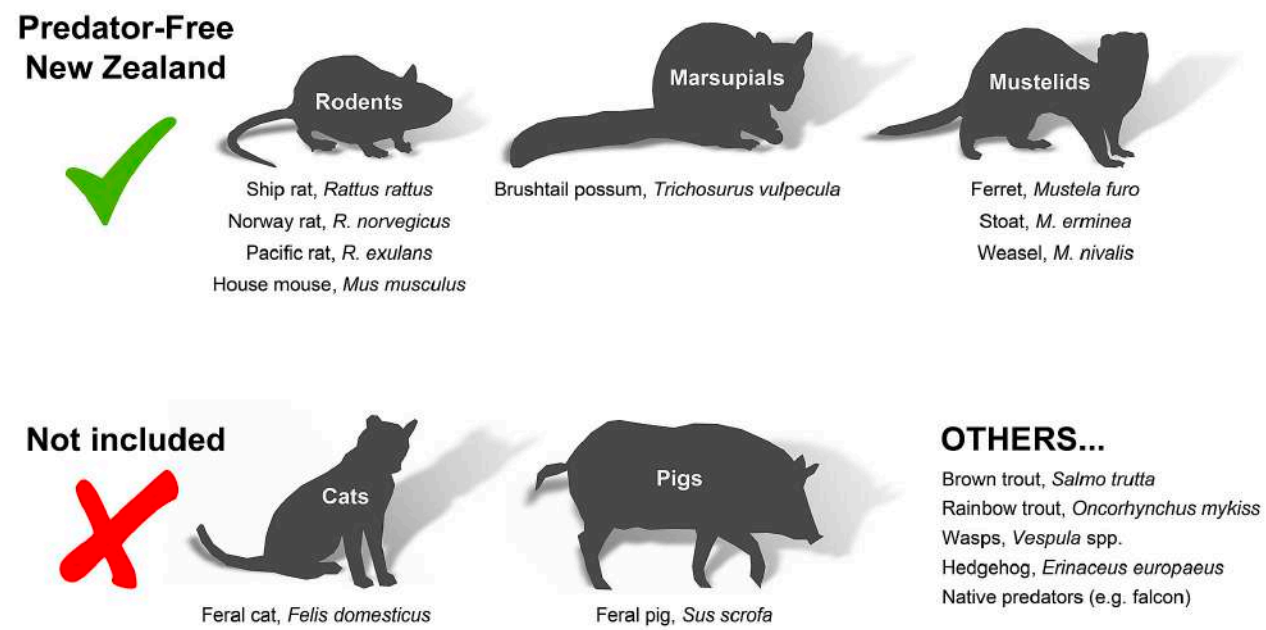
Gene drives

Fighting Lyme Disease in the Genes of Nantucket's Mice

Give this article

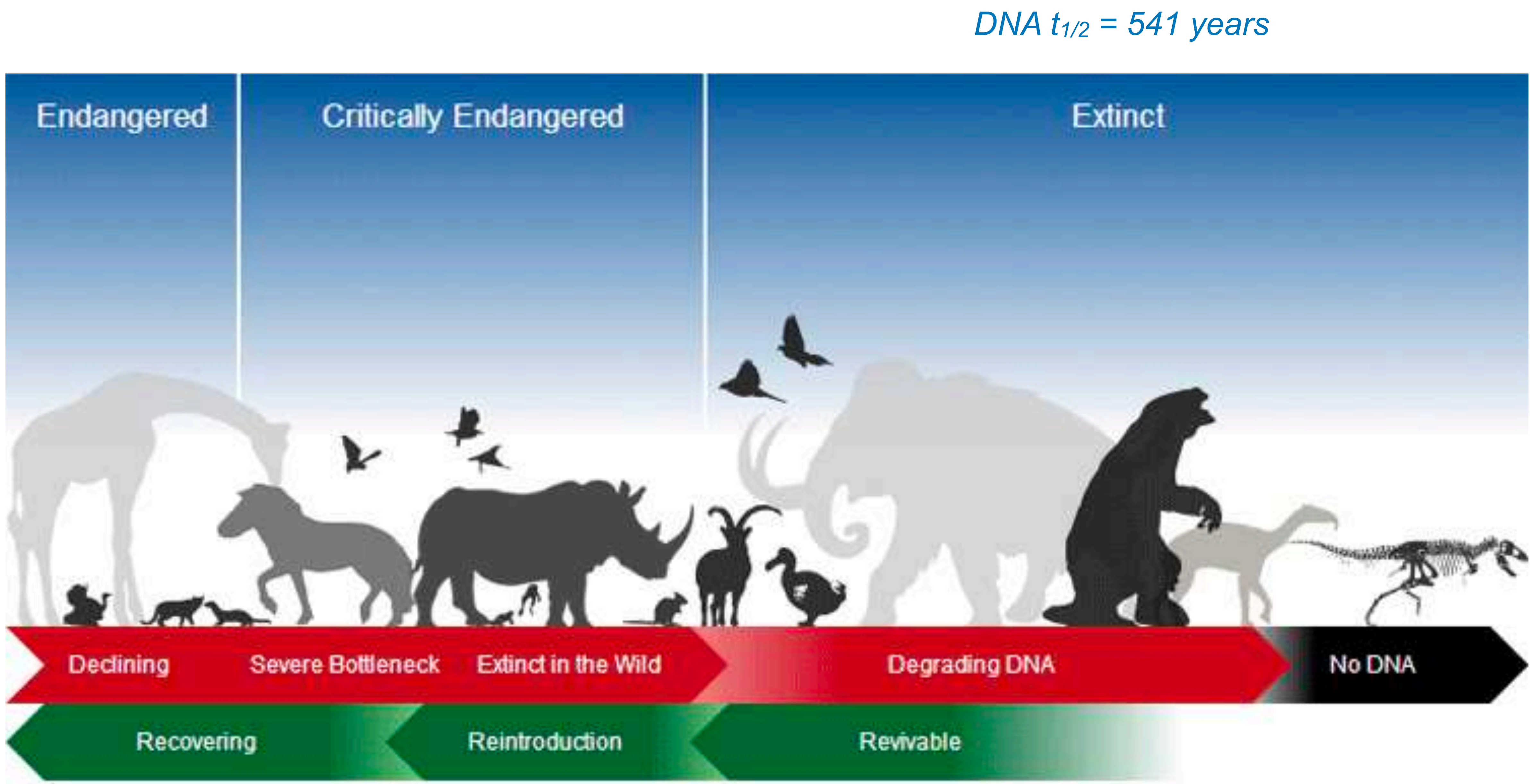
White-footed mice carry the pathogen that causes Lyme disease. An M.I.T. scientist is proposing to create mice that are genetically engineered to break the cycle of transmission. Yousur Al-Hlou/The New York Times

PREDATOR FREE NZ



Nat. Rev. Genetics, **2022,** *23,* 5-22.

De-extinction



Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

De-extinction

Fibres and cellular structures preserved in 75-million-year-old dinosaur specimens

Sergio Bertazzo[1,†], Susannah C.R. Maidment[2], Charalambos Kallepitis[1,3,4], Sarah Fearn[1], Molly M. Stevens[1,3,4] & Hai-nan Xie[1,3,4]

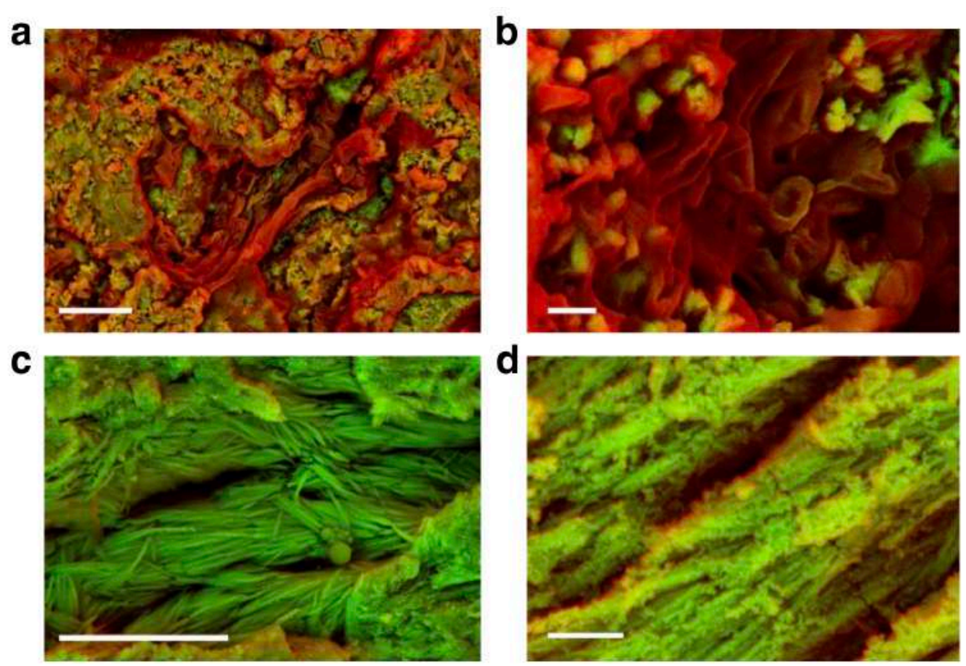
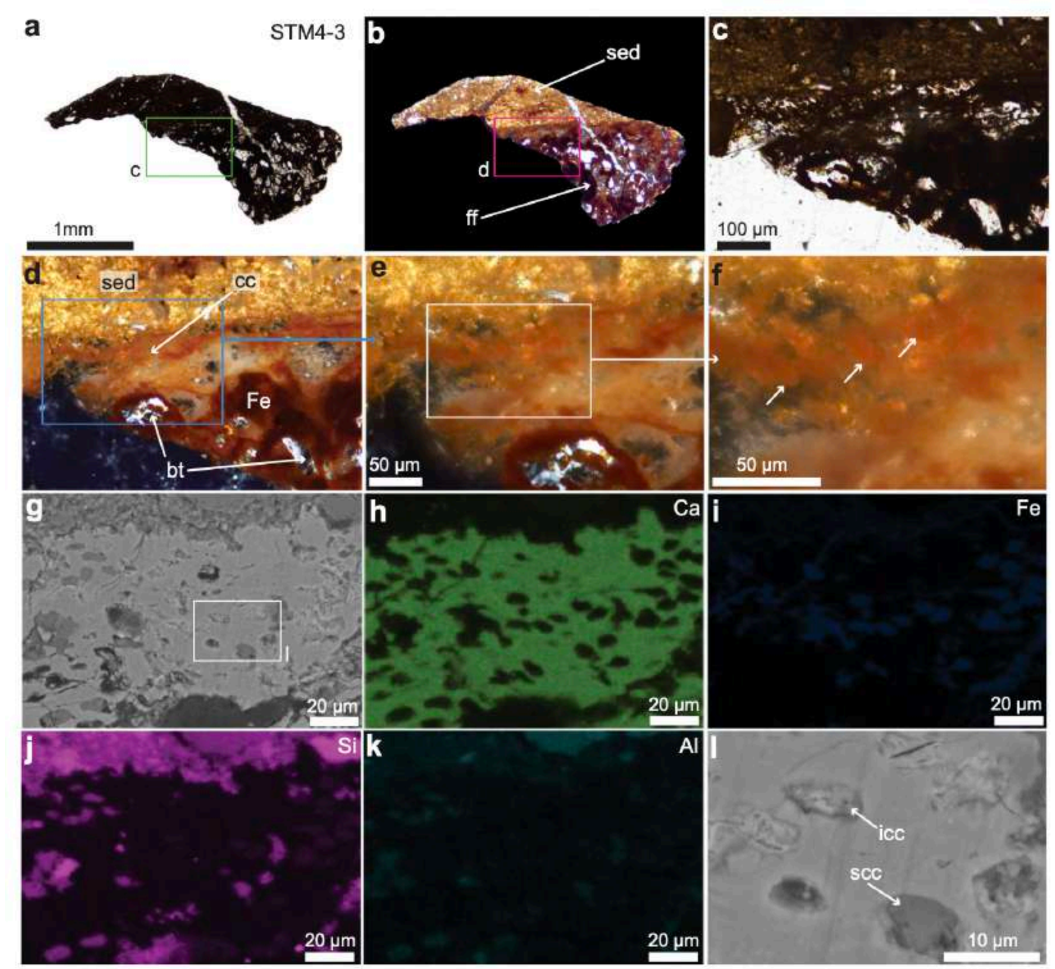


Figure 1 | Density-dependent colour scanning electron micrographs of samples of NHMUK R12562, an ungual claw of an indeterminate theropod dinosaur, and NHMUK R4493, ribs from an indeterminate dinosaur. (**a**) Amorphous carbon-rich material (red) surrounded by dense material (green). Scale bar, 5 μm. (**b**) Erythrocyte-like structures composed of carbon surrounded by cement. Scale bar, 1 μm. For comparison, fixed blood from an emu (Dromaius) is shown in Supplementary Fig. 2c, d. Fibrous structures. Scale bar, 5 μm in (**c**) and 1 μm in (**d**).

Nat. Comm., **2015,** *6*, 7352.

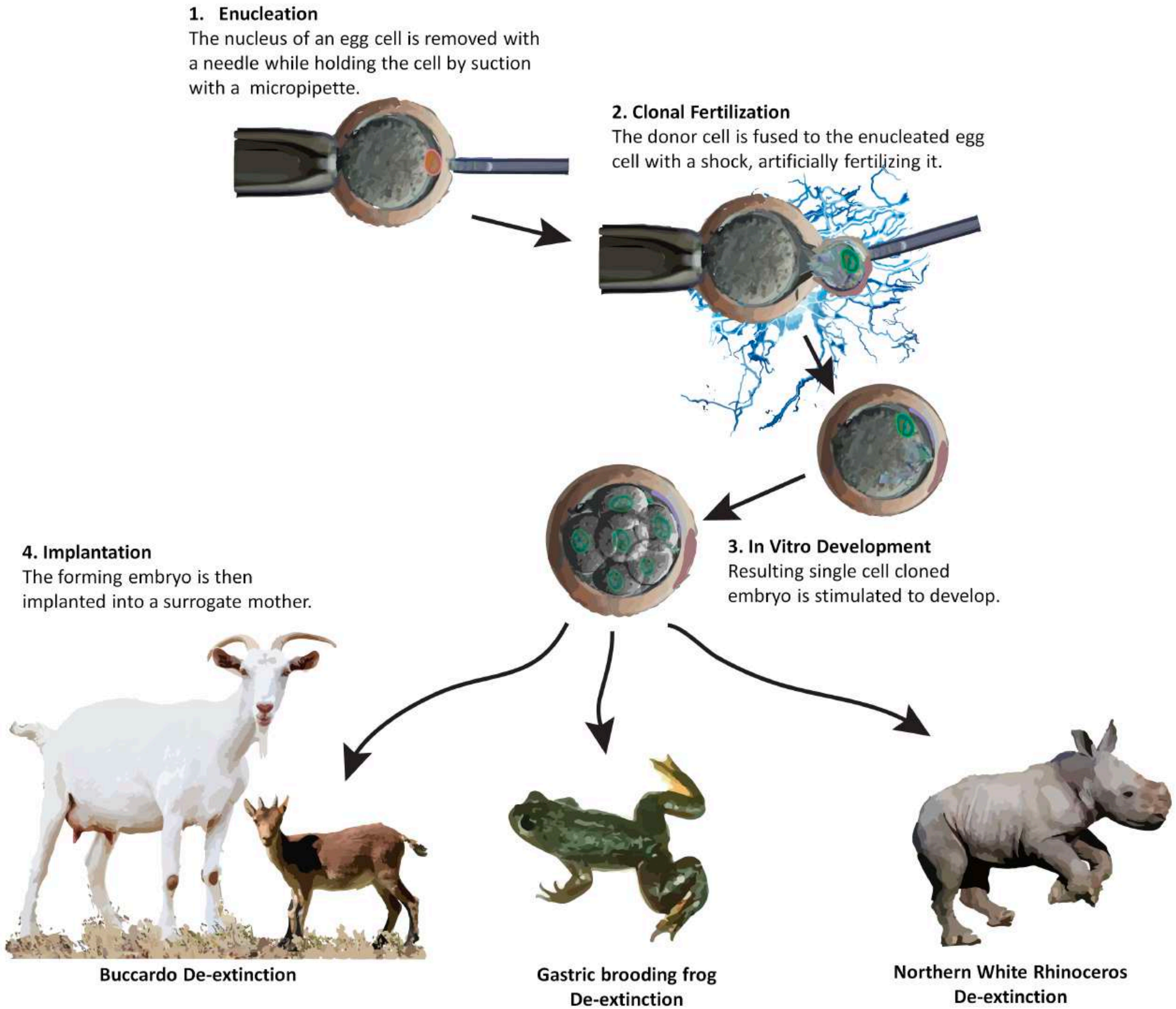
Nuclear preservation in the cartilage of the Jehol dinosaur *Caudipteryx*

Xiaoting Zheng[1,2], Alida M. Bailleul[3,4✉], Zhiheng Li[3,4], Xiaoli Wang[1,2] & Zhonghe Zhou[3,4]



Nat. Comm. Biol, **2021,** *4*, 1125.

De-extinction

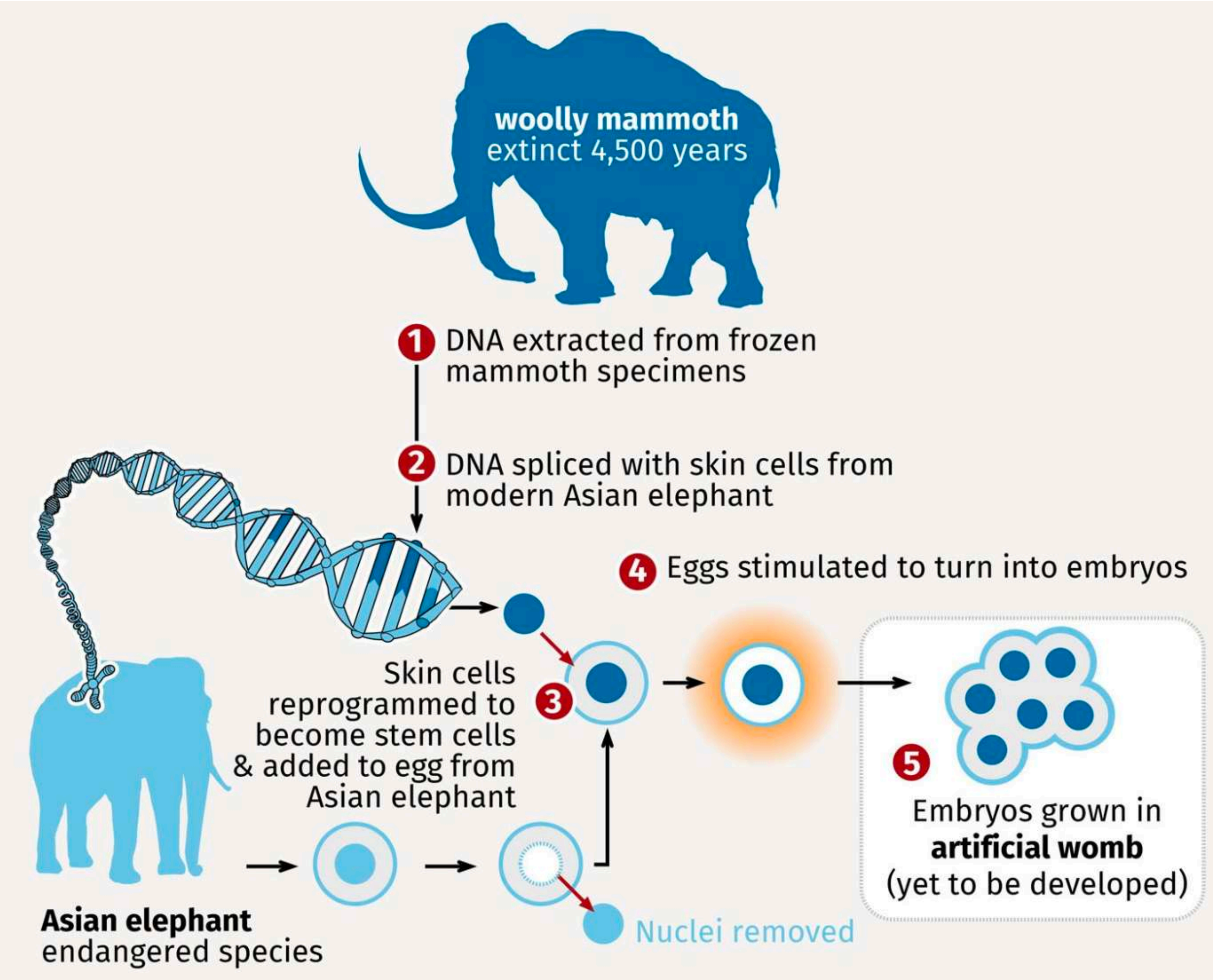


Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

De-extinction

Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

De-extinction



Nat. Rev. Mol. Cell. Biol., **2020,** *21*, 661-677.

Final perspectives

a variety of effective tools and platforms exist for manipulating DNA/RNA

these continue to get better in every way

delivery of CRISPR in vivo remains a challenge

methods continue to get better in every way

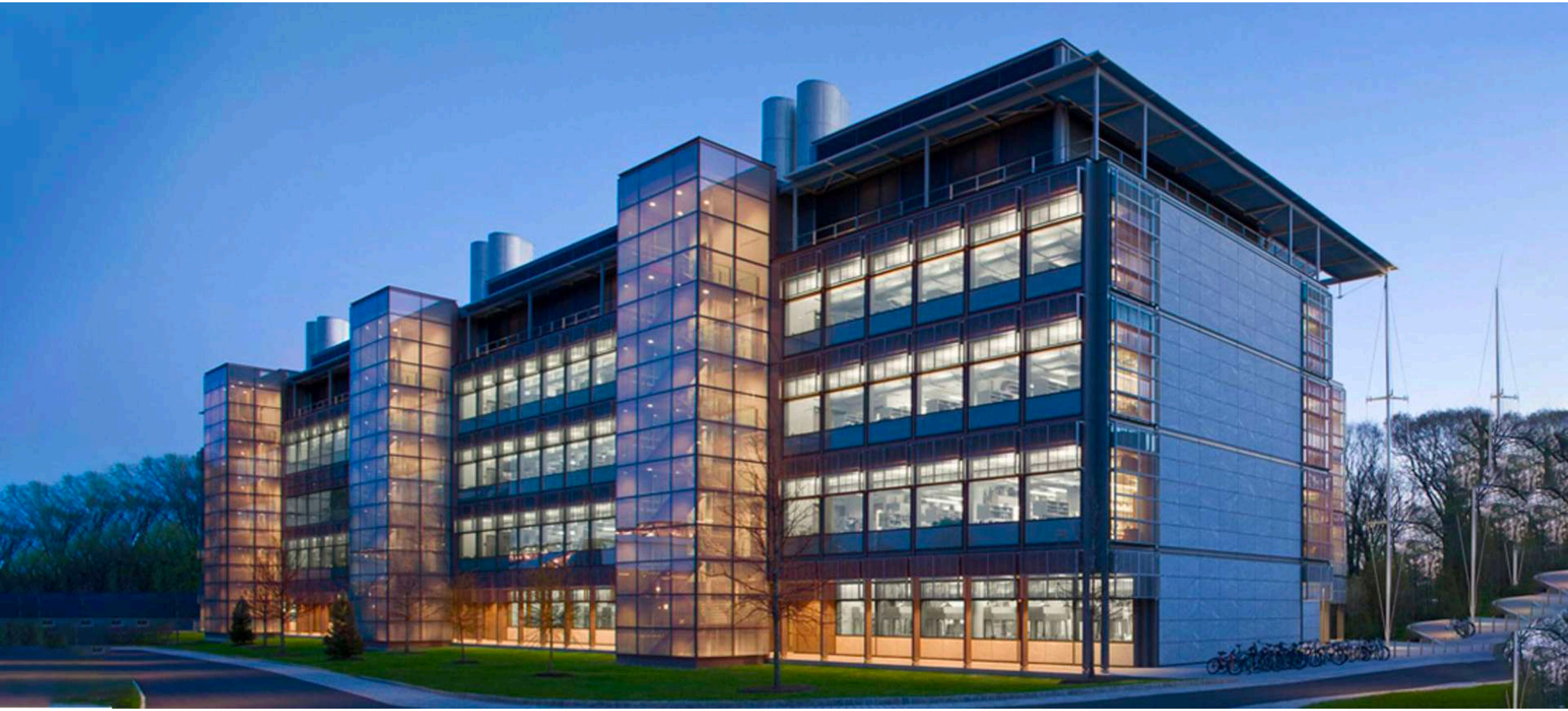
our understanding of genetics is the biggest bottleneck

interplay of multiple genes, environment, etc

ethical debates must be had, and laws must be made

Thank you

Thank you



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