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The Scientific Foundations of Human Genome Editing

Fyodor D. Urnov, PhD

Professor of Molecular Therapeutics,
Department of Molecular and Cell Biology,
University of California, Berkeley

Scientific Director, Innovative Genomics Institute, UC Berkeley

Presentation to the FDA CTGATC | October 31 2023

Fyodor Urnov: disclosures

- Cimeio Therapeutics: SAB chair, paid advisor, hold equity
- Ionis Pharmaceuticals: paid advisor
- Tune Therapeutics: scientific co-founder, paid advisor, hold equity
- Vertex Pharmaceuticals: paid consultant on exa-cel program

Targeted genetic engineering before genome editing

Gene targeting → Inefficient → Genotoxic

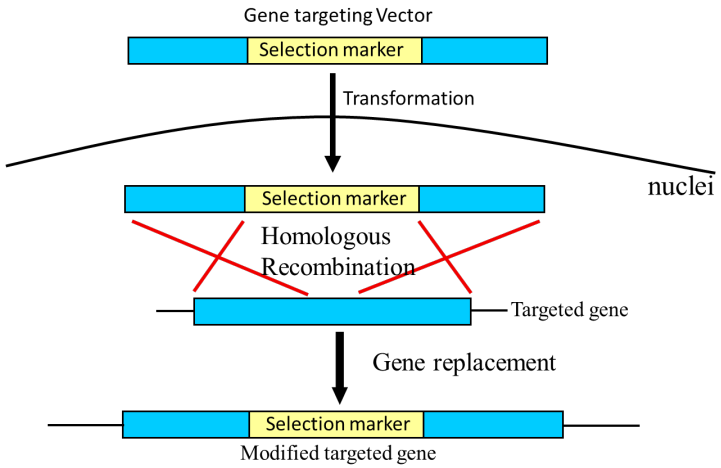
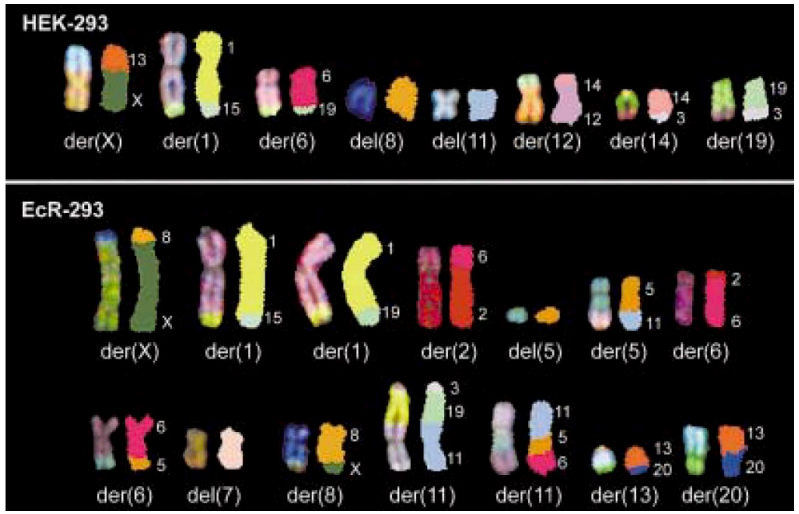


TABLE 1. Summary of gene targeting experiments

Cell line	Gene	Exon(s)	No. of colonies screened[a]	No. of correctly targeted clones[b]	Targeting frequency (%)[c]
NALM-6 (WT)	DNA-PK$_{cs}$	81 to 83	432	1	0.2
HCT116 (WT)	DNA-PK$_{cs}$	1	372	0	0.0



Inapplicable to primary human cells = no therapeutic applications

Ruis et al *Mol Cell Biol* (2008) 28(20):6182-95.

Bylund et al. *Cytogenet Genome Res* (2004) 106 (1): 28–32

Genome Editing: a Whole New World

"Cas9" in PubMed:
27,300 references
T cells, HSPCs,
liver, eye etc

The genome editing toolbox

`

1994 2001-2005 2012 2023

Maria Jasin | Dana Carroll, Matthew Porteus, David Baltimore, Sangamo, others | Jennifer Doudna, Emmanuelle Charpentier

Genome Editing:
From Basic Science to Universal Tool

"Cas9" in PubMed:
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The genome editing toolbox

Exponential scaleup

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]‡

Genome editing with Cas9

1994 2001-2005 2012 2023

Maria Jasin | Dana Carroll, Matthew Porteus, David Baltimore, Sangamo, others | Jennifer Doudna, Emmanuelle Charpentier

Genome Editing:
Three Decades of Scientific Insight

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

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Genome editing with Cas9

toolbox

Introduction of Double-Strand Breaks into the Genome of Mouse Cells by Expression of a Rare-Cutting Endonuclease

PHILIPPE ROUET, FATIMA SMIH, AND MARIA JASIN*

Cell Biology and Genetics Program, Sloan-Kettering Institute and Cornell University Graduate School of Medical Sciences, New York, New York 10021

Genome editing native genes

principle

DSB drives edits

1994 2001-2005 2012 2023

Maria Jasin | Dana Carroll, Matthew Porteus, David Baltimore, Sangamo, others | Jennifer Doudna, Emmanuelle Charpentier

Genome Editing B.C. (Before CRISPR): Two Enduring Concepts

2005

2010

Genome editing with engineered zinc finger nucleases

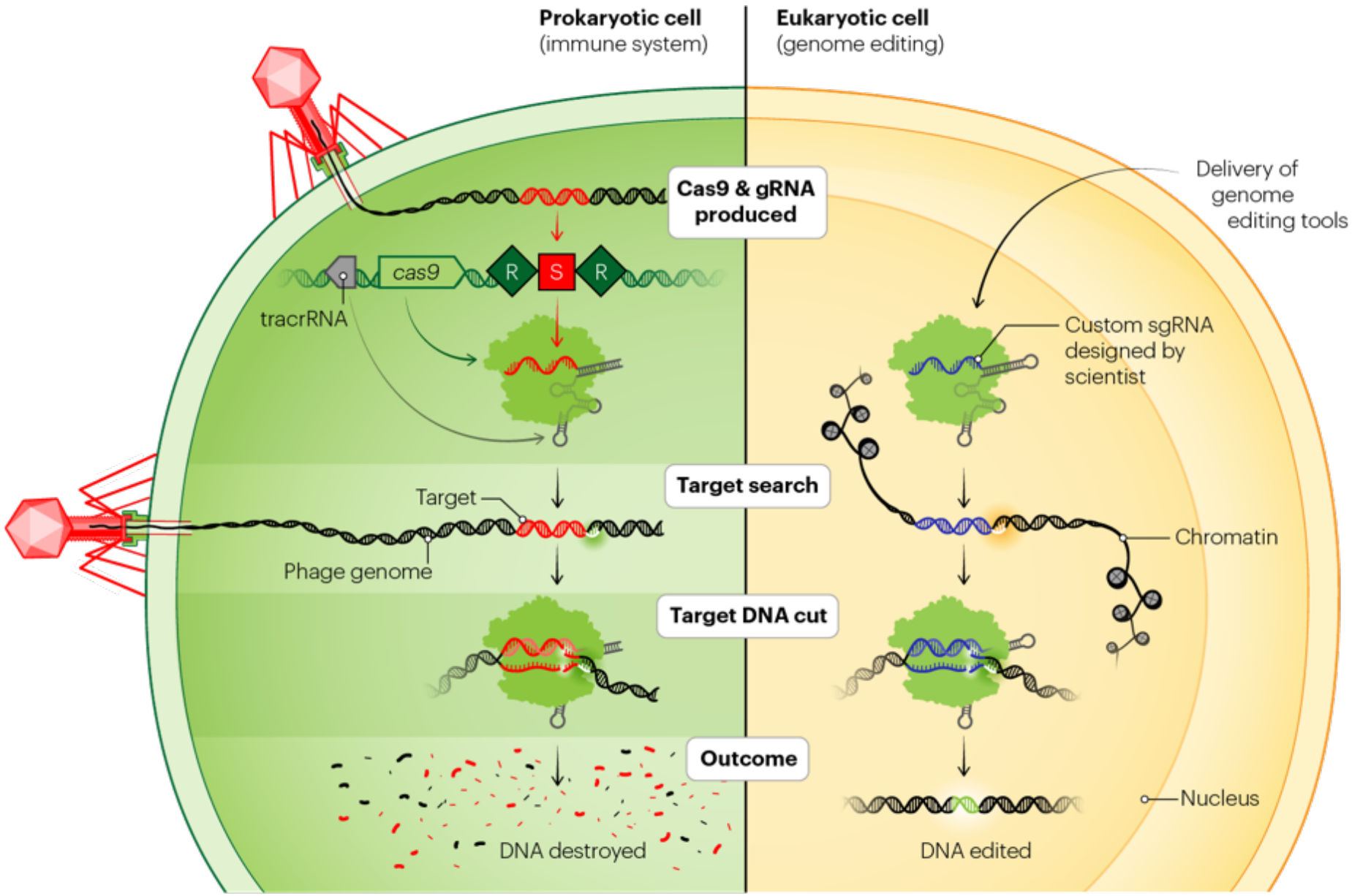
An engineered **enzyme** ("the genome editor")
(i) binds a DNA target in a cell in an investigator-specified way and
(ii) drives an enzymatic reaction that results in genetic change at that target.

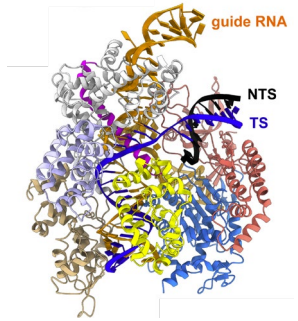
1 – as all enzymes, genome editors follow biochemical principles that can be studied, understood, and that inform their in-cell action

2 – in contrast to enzymes reacting with substrates in a test tubes, genome editors act on the genome in its living form.

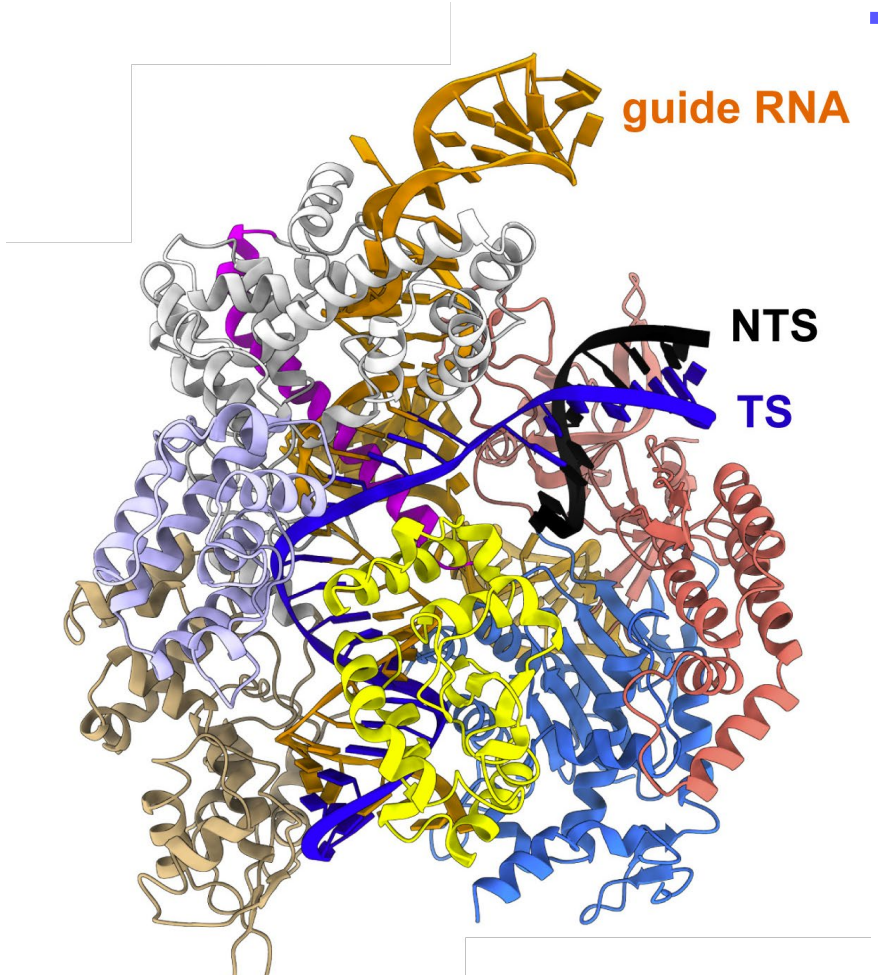
The biology of the cell is the prism through which genome editors act.

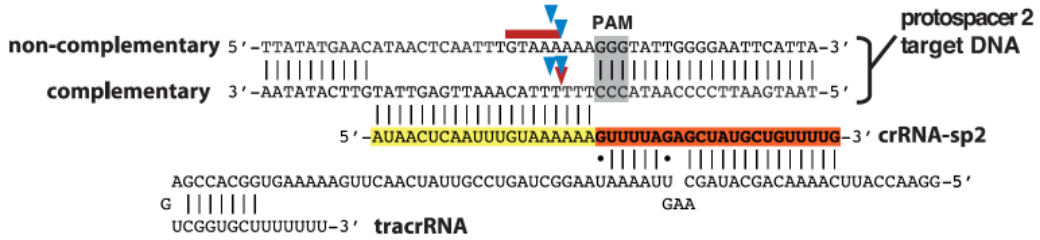
Urnov et al *Nature* (2005) 435: 646-651 | Urnov et al *Nature Reviews Genetics* (2010) 11: 636-646.

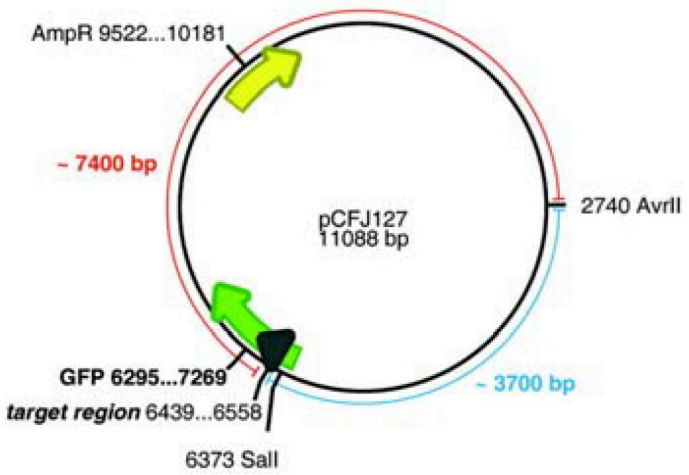


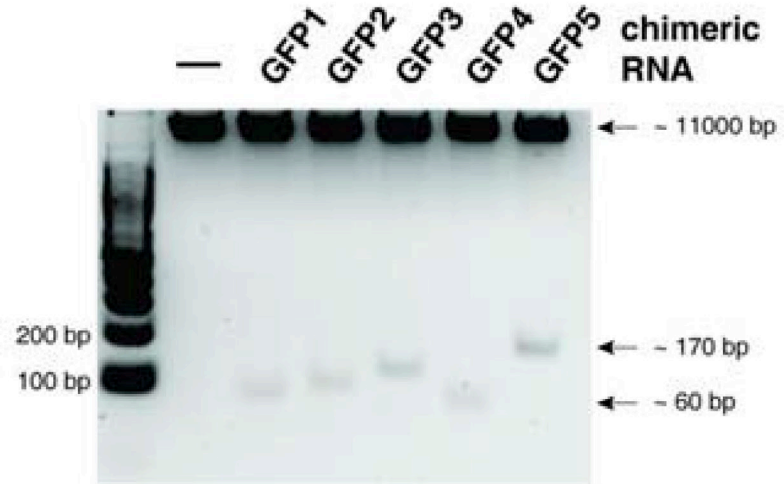


Innovative Genomics Institute | CRISPRpedia



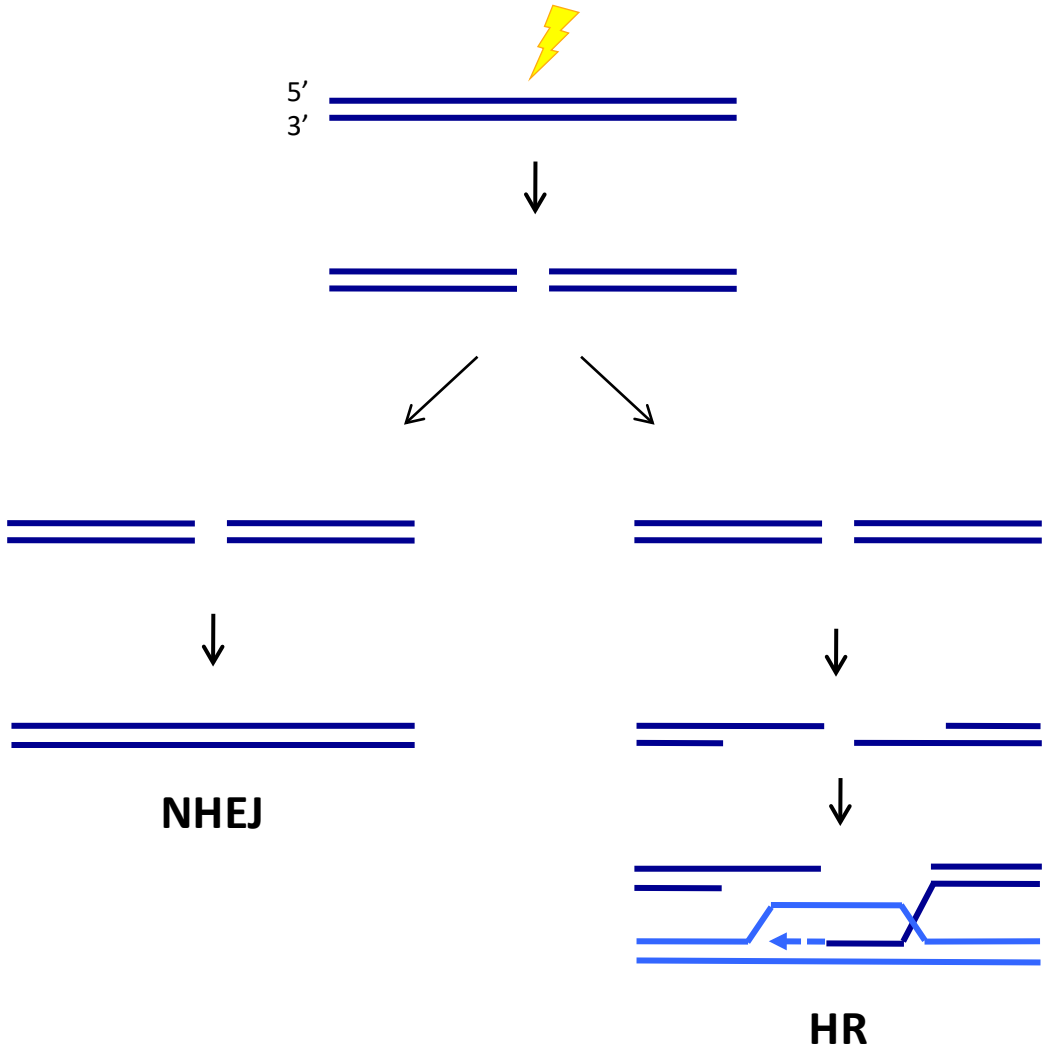






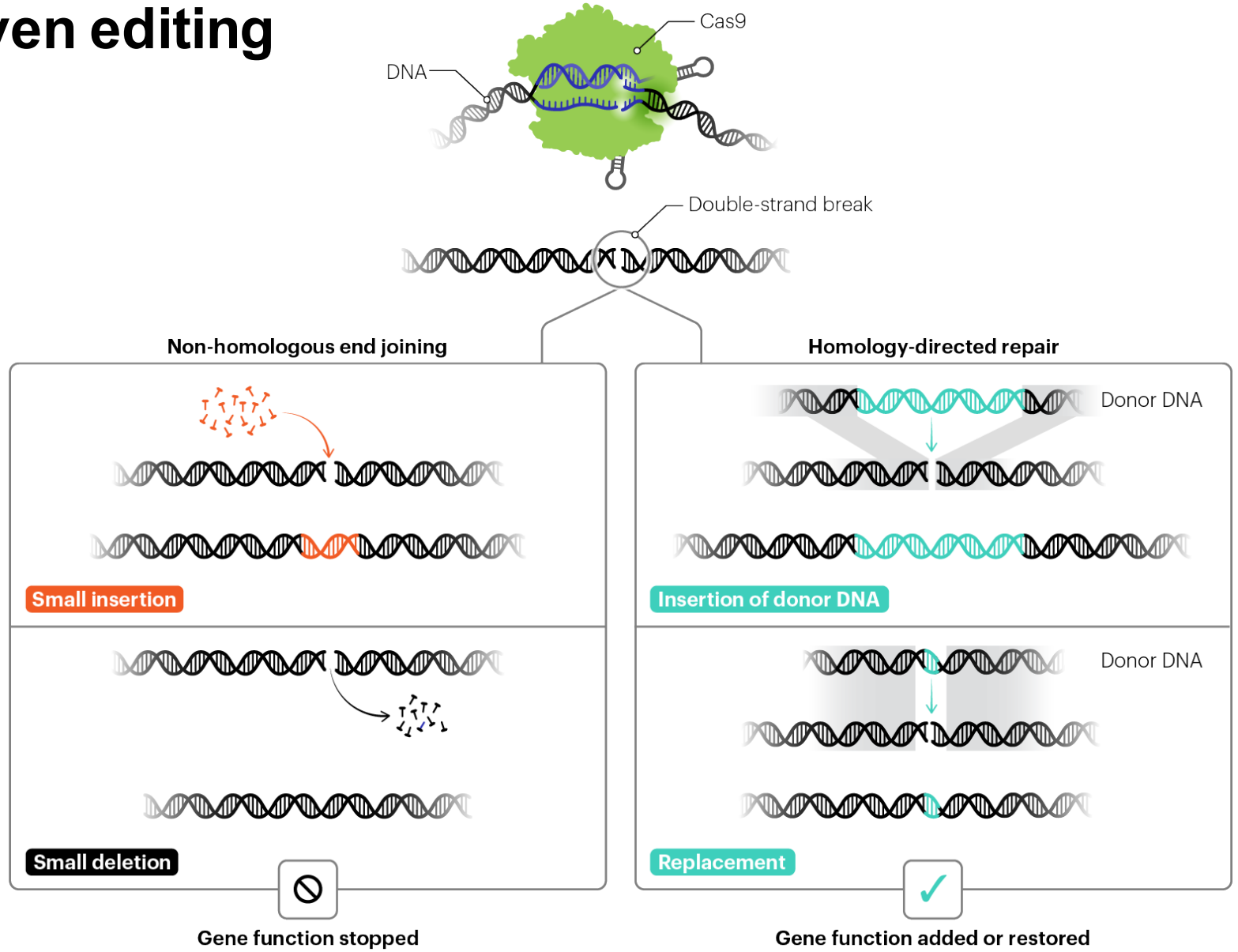
Jinek et al *Science* (2012) 337: 816-821 | Pacesa et al *Cell* (2022) 185: 4067-4081.

Double Strand Break (DSB) Repair: Two Major Pathways



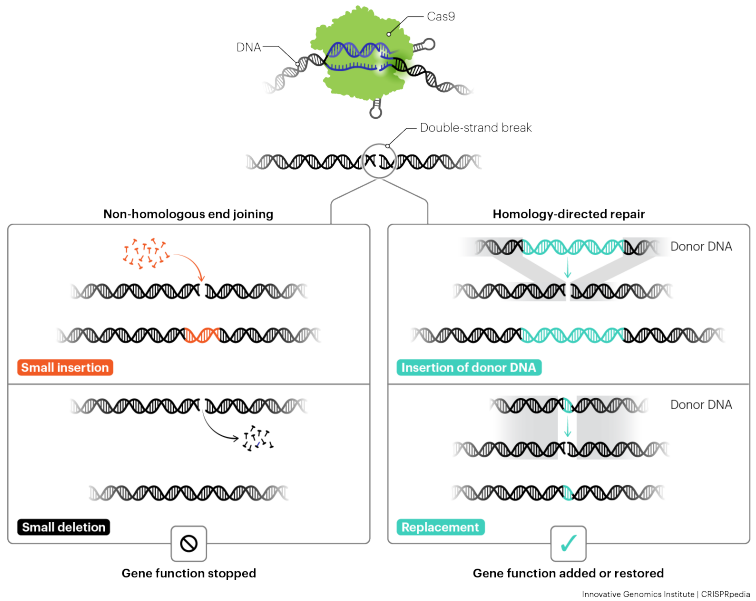
Schematic provided by Dr Lorraine Symington, Columbia University

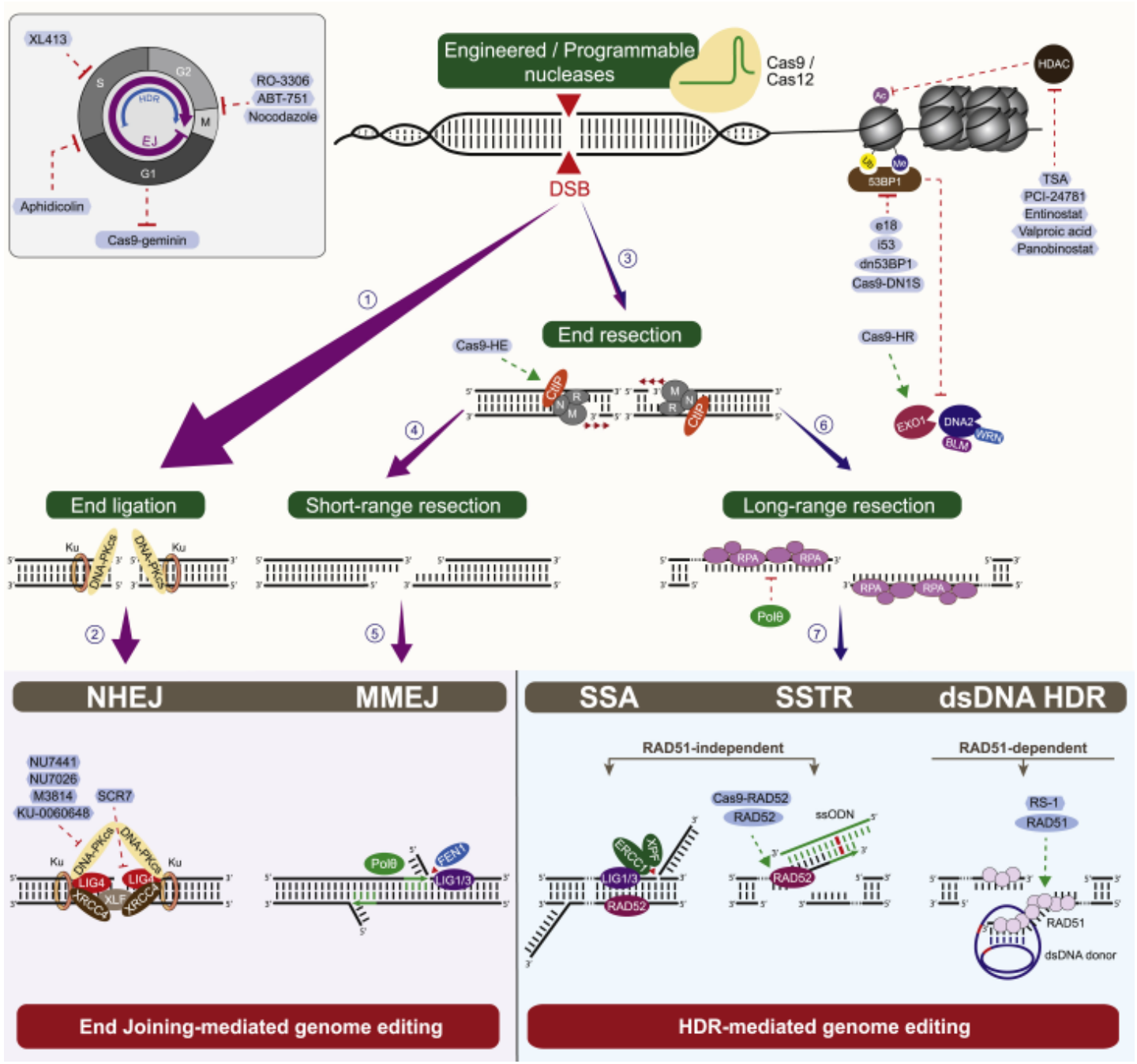
DSB-driven editing



https://innovativegenomics.org/crisprpedia/

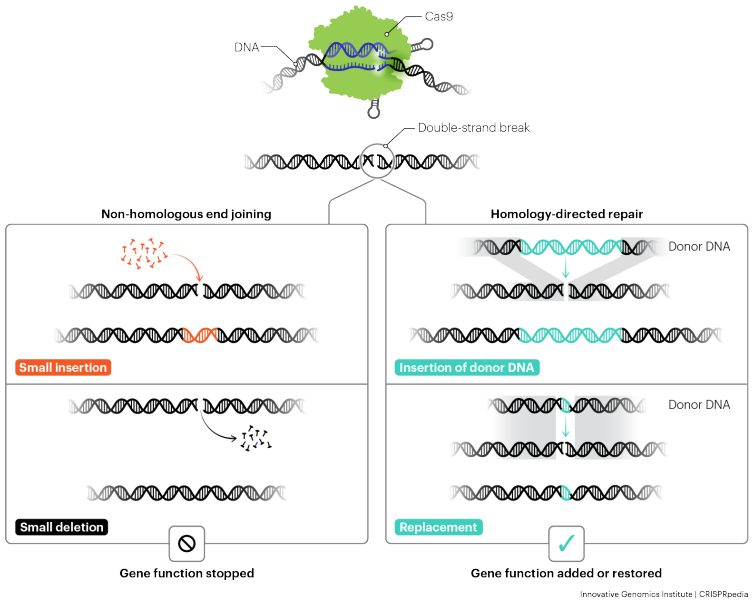
DSB-driven editing and DSB-R

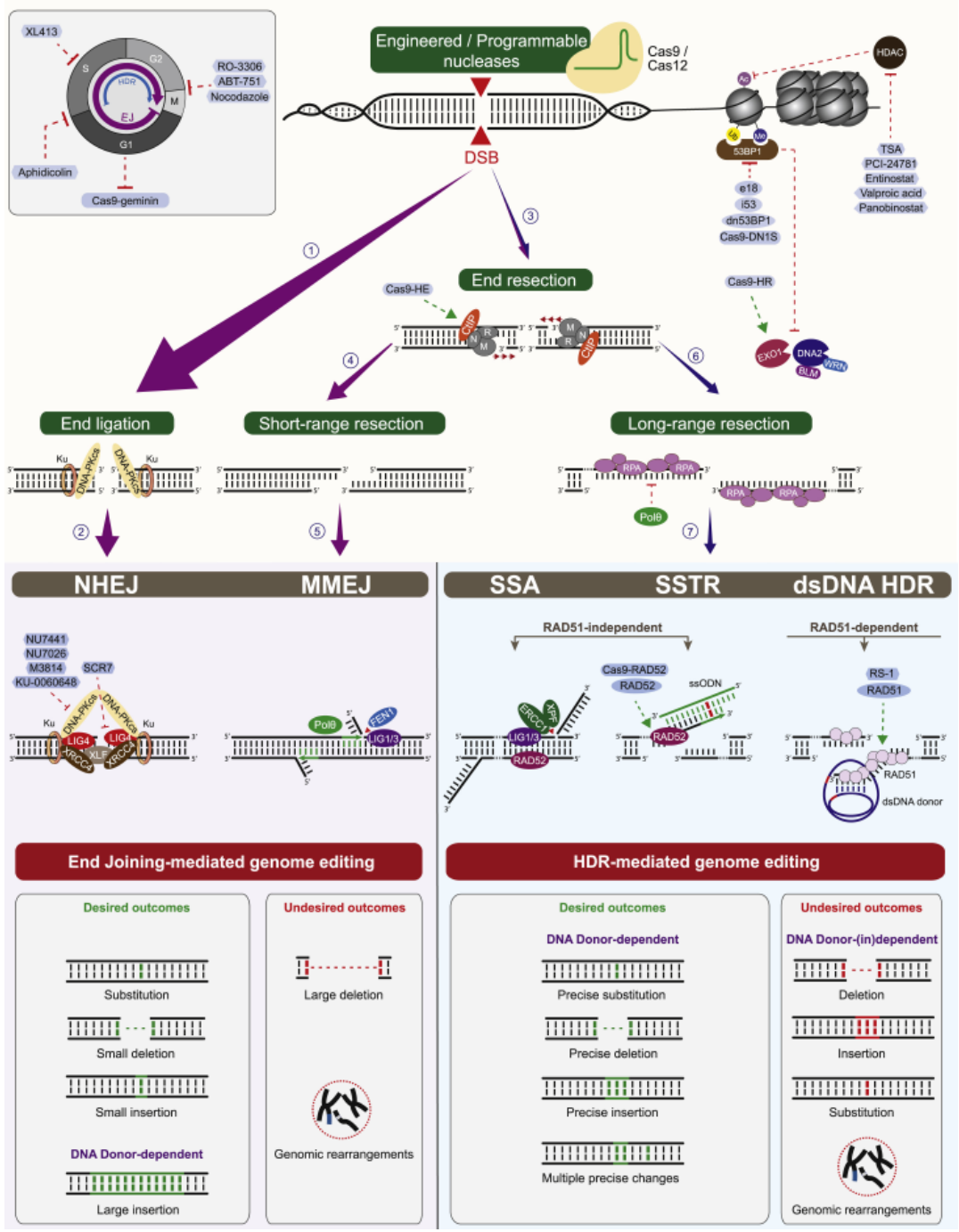




Nambiar et al. (2022) *Molecular Cell* 82: 348-388

DSB-driven editing and DSB-R



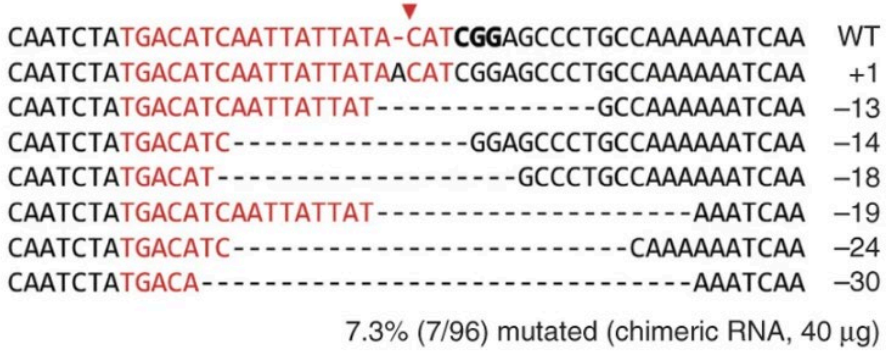


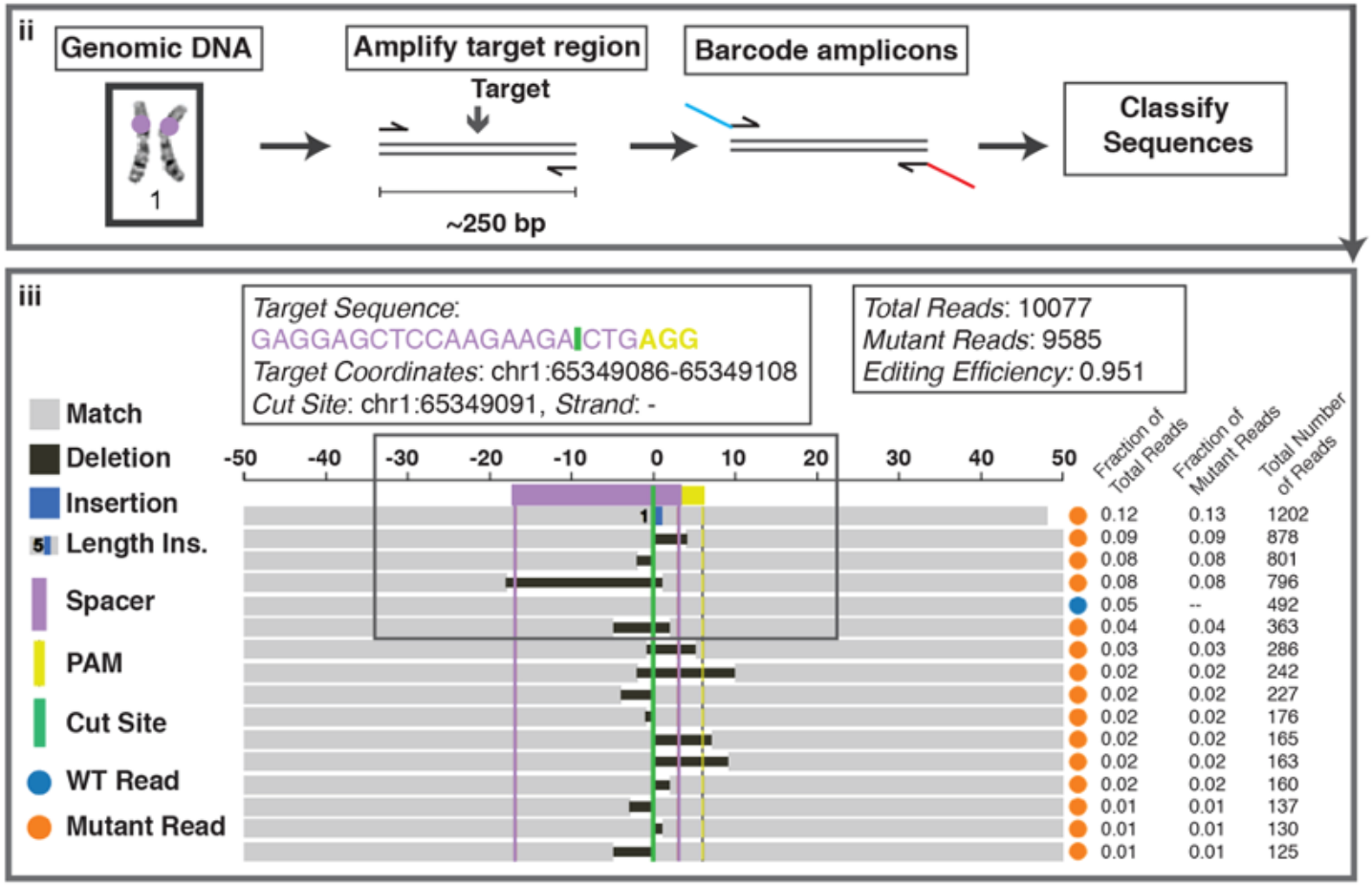
Nambiar et al. (2022) *Molecular Cell* 82: 348-388

Genome editing can produce small, nonrandom deletions and insertions at native genes in human cells

2008 (ZFNs):

2013 (Cas9):

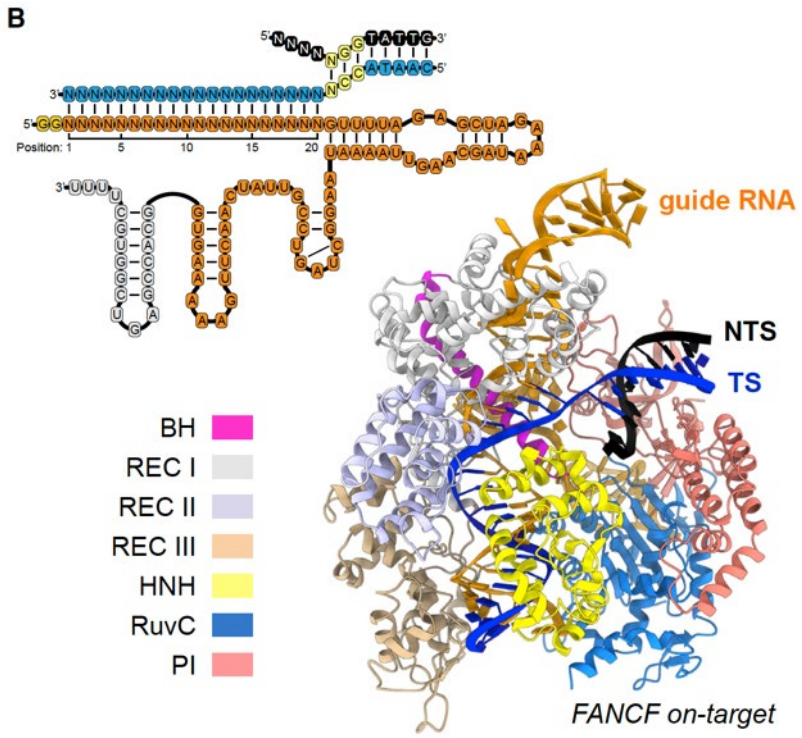




Perez et al. (2008) *Nature Biotechnology 26*: 806-816 | Cho et al (2013) *Nature Biotechnology* 31: 230-232 | van Overbeek et al (2014) *Molecular Cell* 56: 333-339

“Structural basis for Cas9 off-target activity”

“The target DNA specificity of the CRISPR-associated genome editor nuclease Cas9 is determined by complementarity to a 20-nucleotide segment in its guide RNA. However, Cas9 can bind and cleave partially complementary off-target sequences, which raises safety concerns for its use in clinical applications.”



FANCF	guide RNA	G	G	G	A	A	U	C	C	C	U	U	C	U	G	C	A	G	C	A	C	C			
FANCF	on-target		C	C	T	T	A	G	G	G	A	A	G	A	C	G	T	C	G	T	G	G	A	C	C
FANCF	off-target1		·	·	·	·	G	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	T	C	C
FANCF	off-target2		·	·	·	C	·	·	·	·	·	G	·	·	T	·	·	·	·	·	·	·	T	C	C

Pacesa et al *Cell* (2022) 185: 4067-4081.

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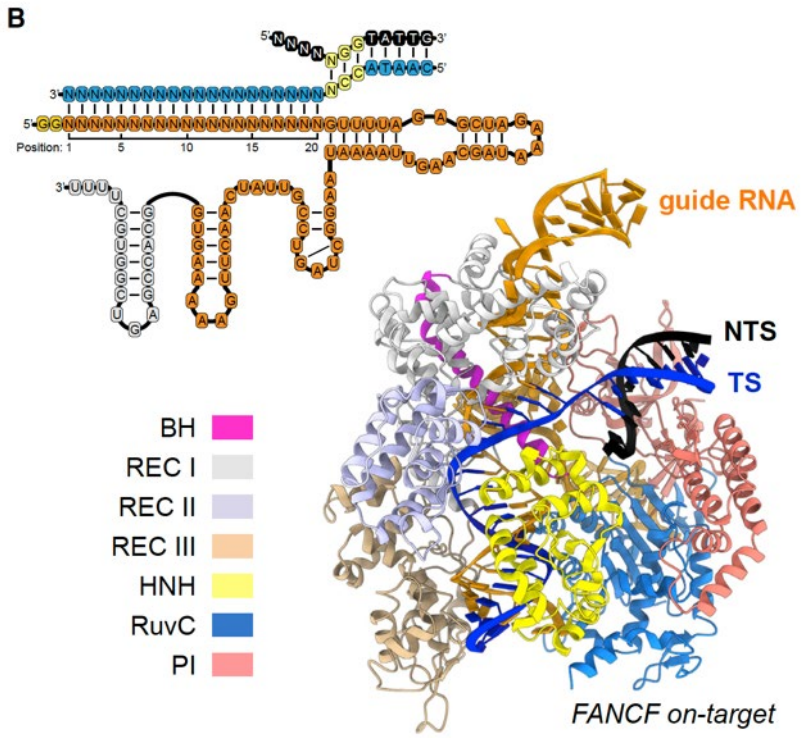
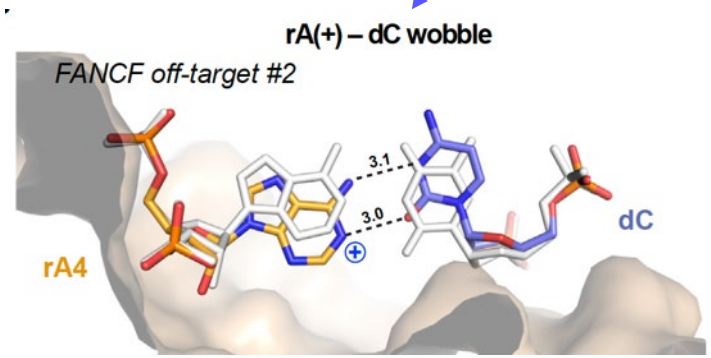


FANCF	guide RNA	G	G	G	A	A	U	C	C	C	U	U	C	U	G	C	A	G	C	A	C	C			
FANCF	on-target		C	C	T	T	A	G	G	G	A	A	G	A	C	G	T	C	G	T	G	G	A	C	C
FANCF	off-target1		·	·	·	·	G	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	T	C	C
FANCF	off-target2		·	·	·	C	·	·	·	·	·	G	·	·	T	·	·	·	·	·	·	·	T	C	C



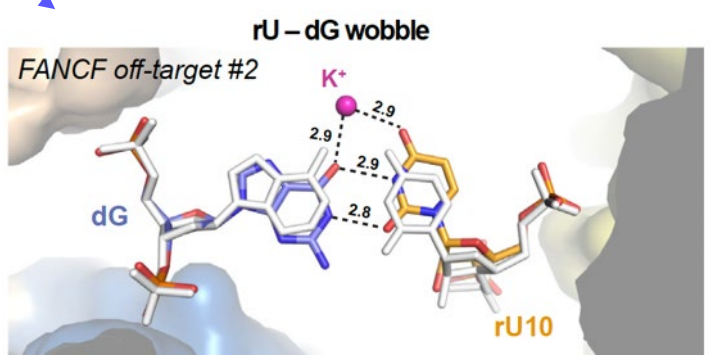


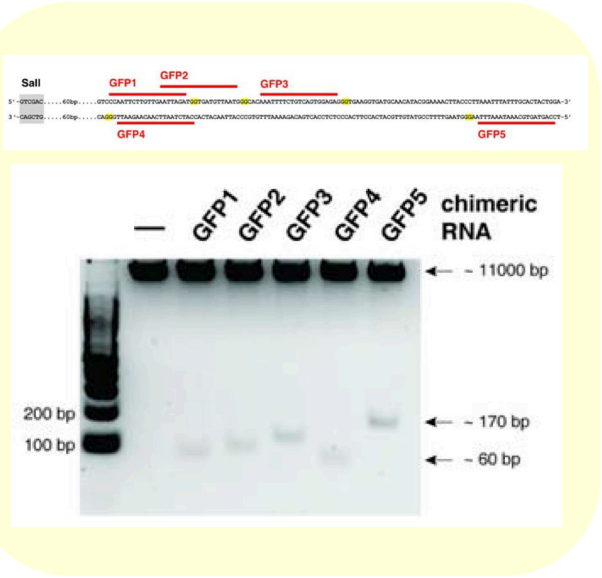
Table 1. Kinetic and thermodynamic analysis of off-target substrate binding and cleavage

Gene	Target	24-h cleavage (%)	k_{obs} (min^{-1})	k_{on} ($M^{-1}. s^{-1}$)	k_{off} (s^{-1})	K_d (pM)
FANCF	on-target	97.5	0.238 ± 0.013	3.45 ± 0.19 × 10^6	7.46 ± 0.97 × 10^{-5}	21.6 ± 3.1
FANCF	off-target #1	35.1	0.001 ± 0.0001	3.97 ± 0.06 × 10^6	2.09 ± 0.06 × 10^{-3}	528 ± 17
FANCF	off-target #2	62.4	0.001 ± 0.0002	1.42 ± 0.03 × 10^6	2.45 ± 0.06 × 10^{-3}	1,730 ± 60

Pacesa et al *Cell* (2022) 185: 4067-4081.

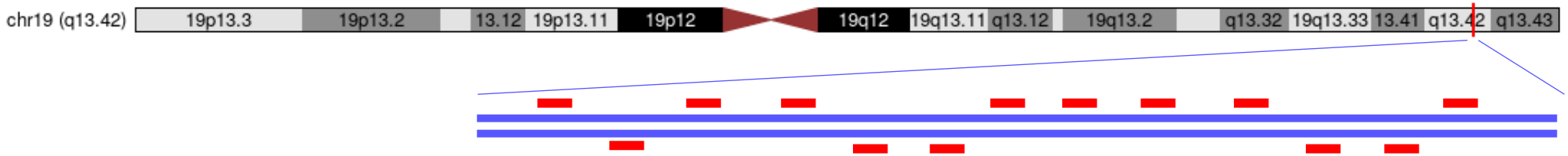
In a test tube:
Cas9 cuts different DNA targets with comparable efficiency

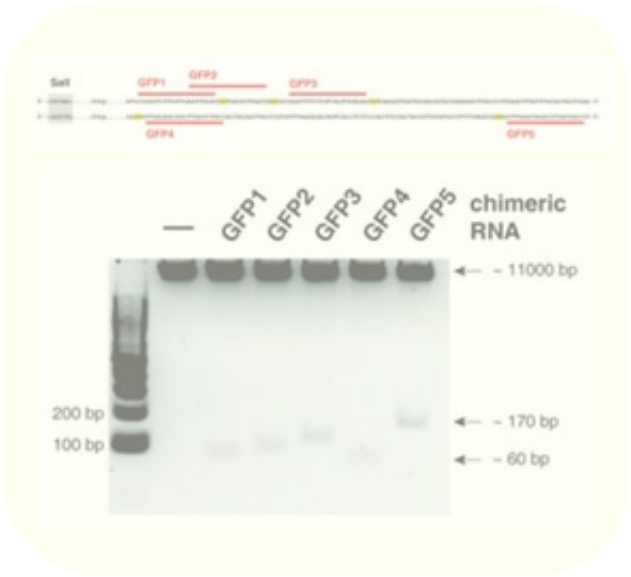
Test tube:

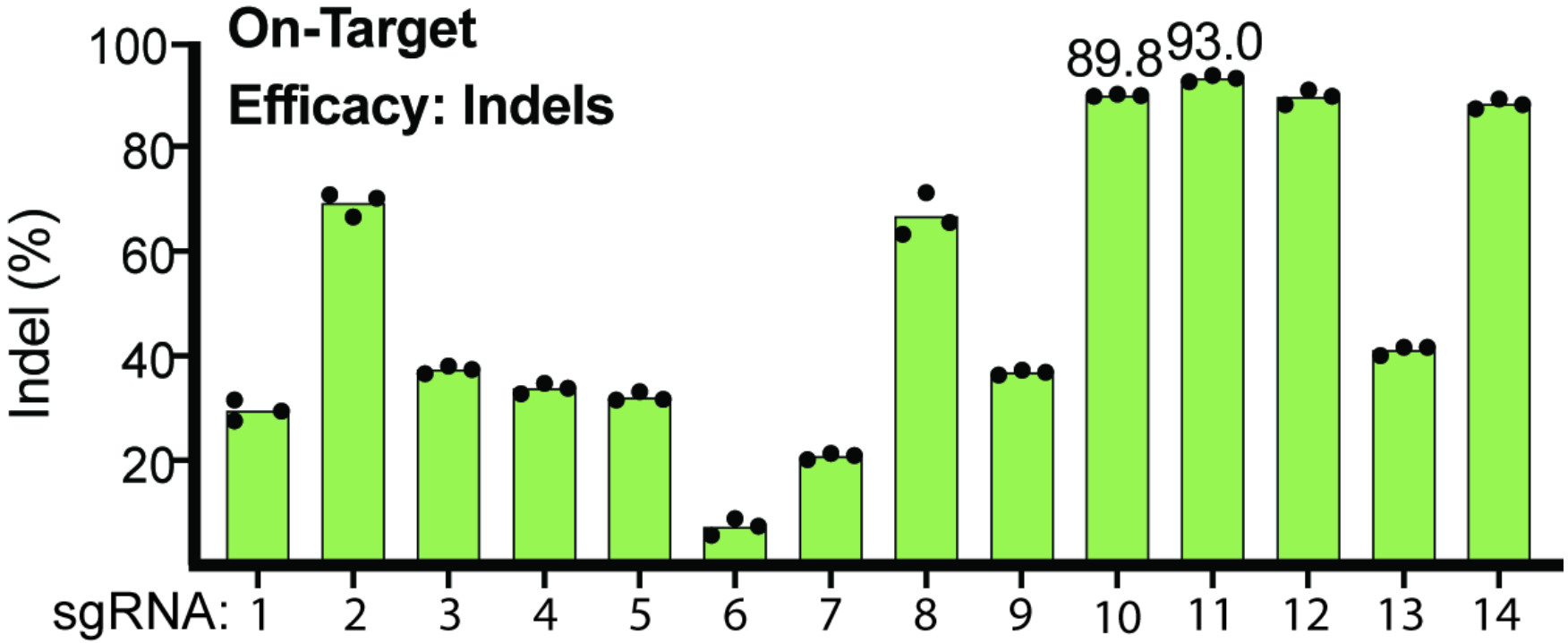


Jinek et al *Science* (2012) 337: 816-821 | Lazzarotto et al *Nature Biotechnology* (2022) 38: 1317-1327.

In a cell:
Cas9 cutting efficiency varies dramatically target-to-target

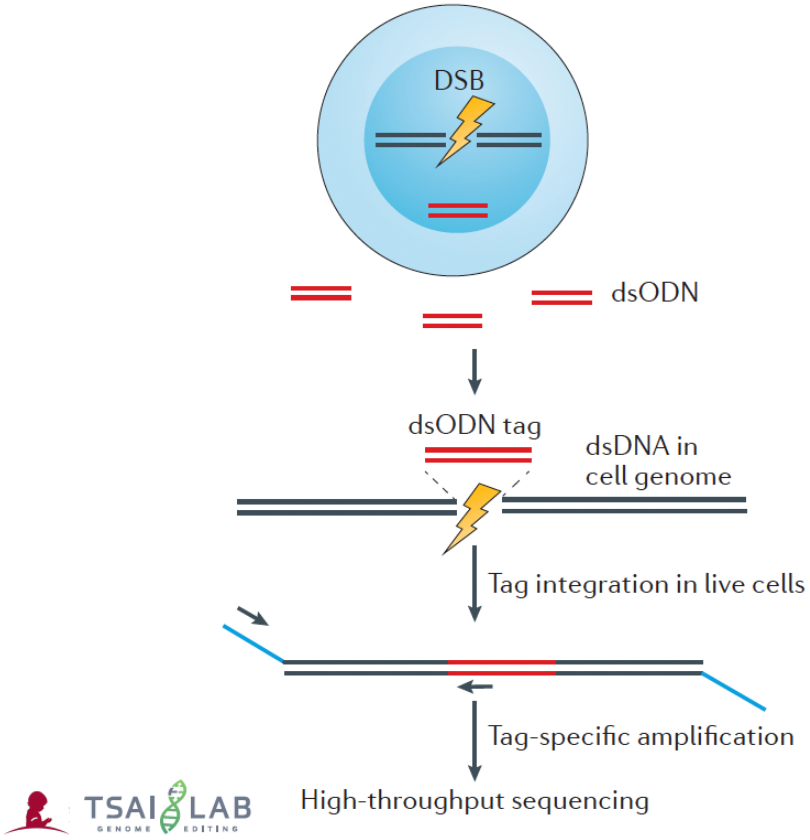


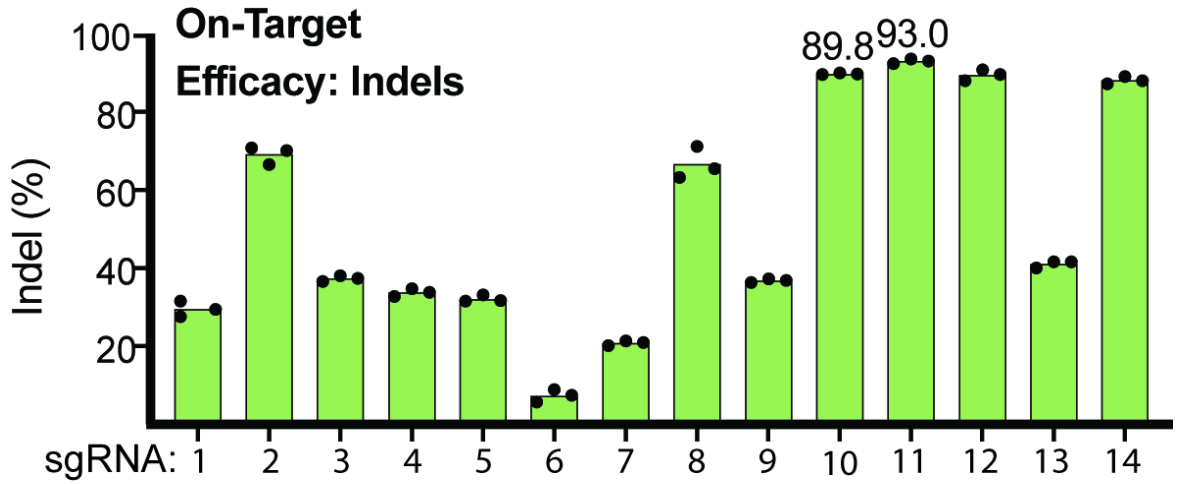


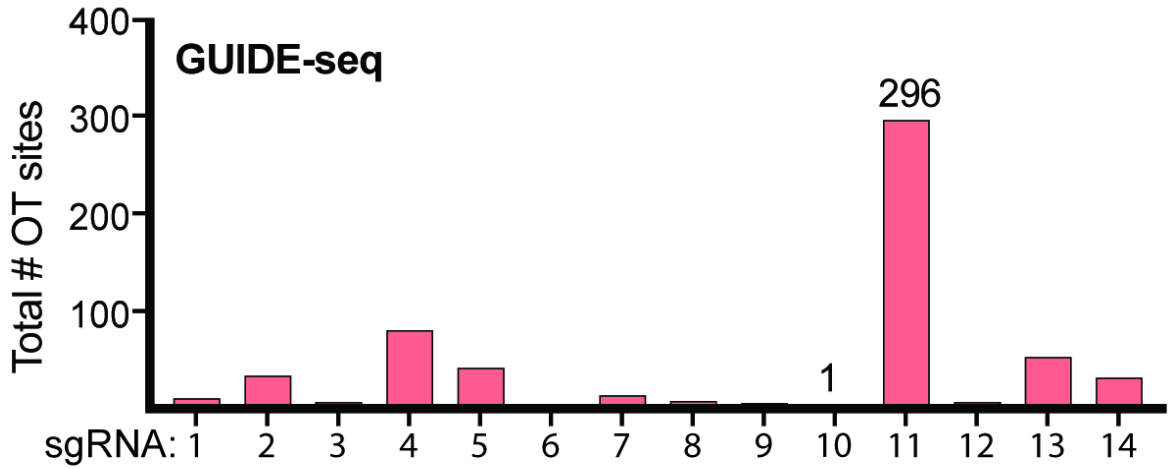


Jinek et al *Science* (2012) 337: 816-821 | Lazzarotto et al *Nature Biotechnology* (2022) 38: 1317-1327.

In a cell:
Cas9 cutting specificity varies dramatically gRNA to gRNA

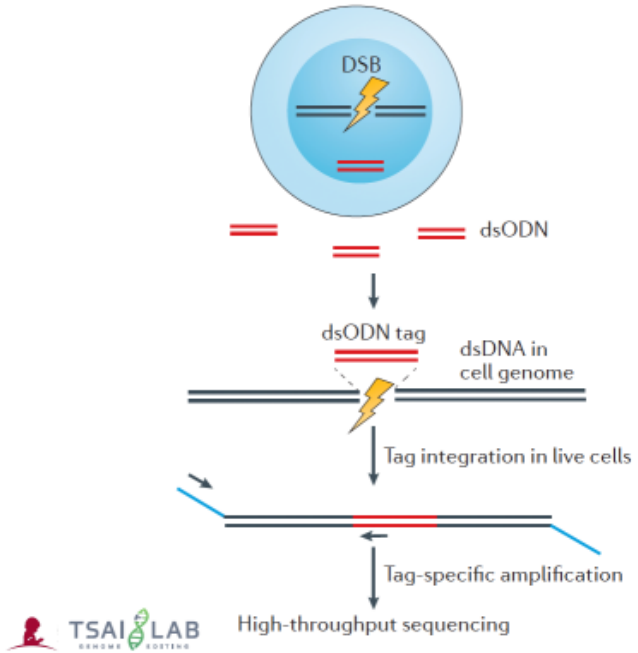


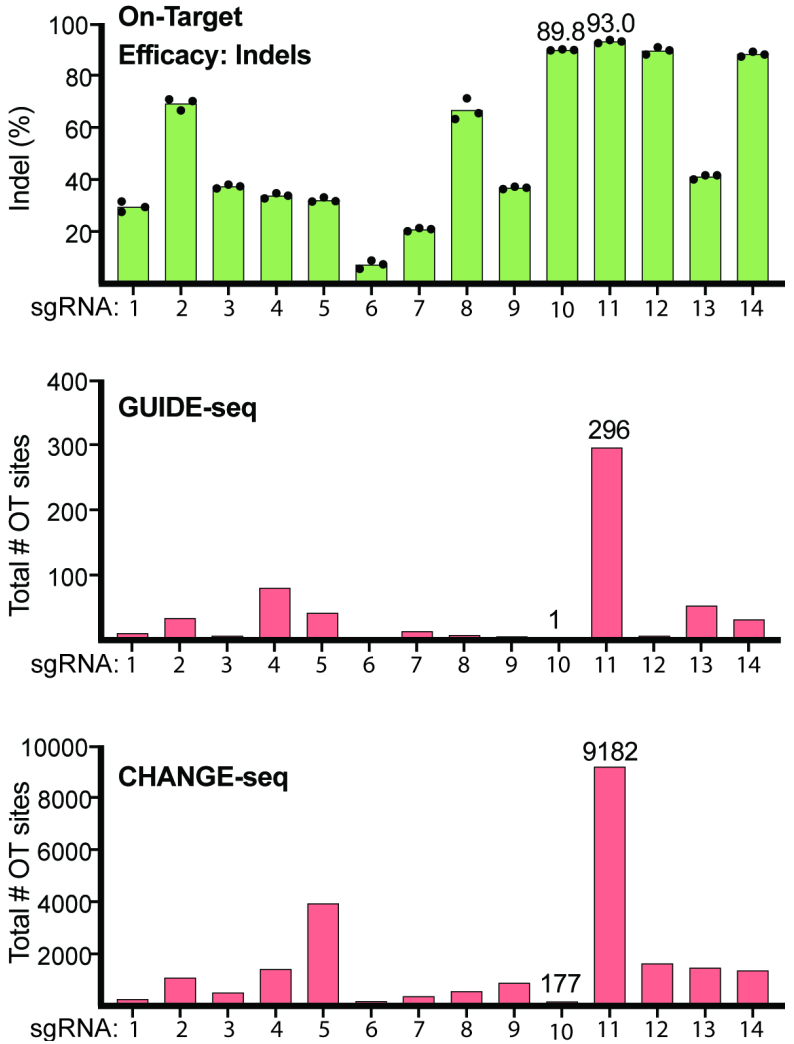


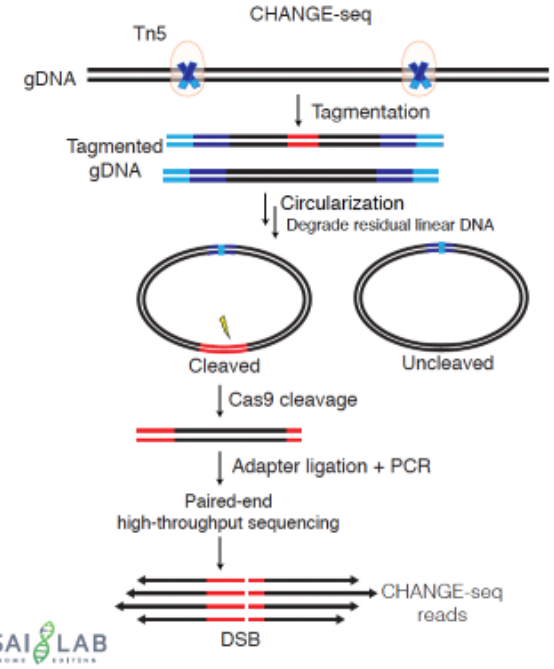


Jinek et al *Science* (2012) 337: 816-821 | Lazzarotto et al *Nature Biotechnology* (2022) 38: 1317-1327 | schematic courtesy of Dr Shengdar Tsai, St Jude

The number of DNA targets a given Cas9-gRNA can cut in the naked human genome is a small fraction of what it actually cuts in a cell

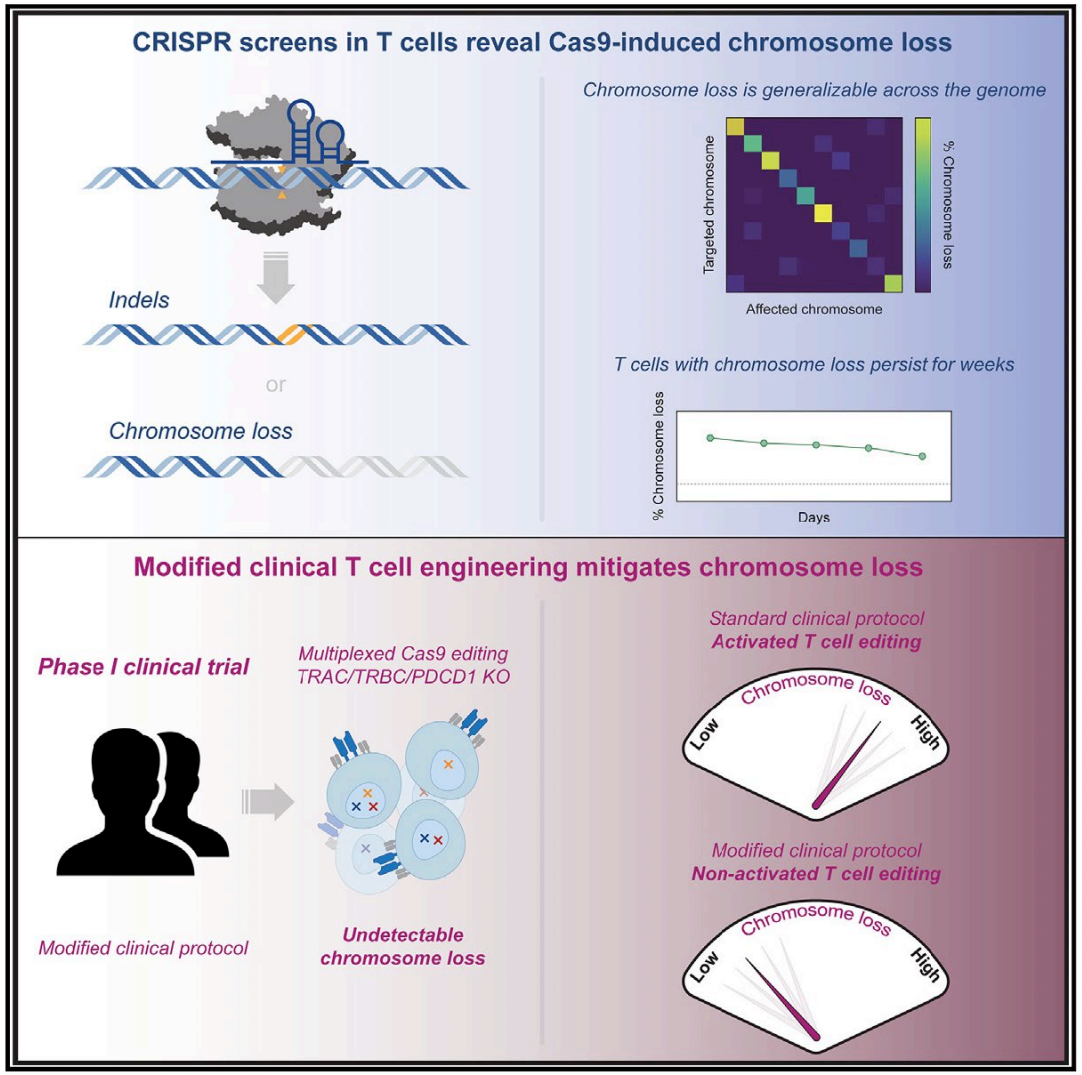






Jinek et al *Science* (2012) 337: 816-821 | Lazzarotto et al *Nature Biotechnology* (2022) 38: 1317-1327 | schematics courtesy of Dr Shengdar Tsai, St Jude

How You Handle the Cells During Genome Editing Provides Critical Input to the Outcome



Article

Mitigation of chromosome loss in clinical CRISPR-Cas9-engineered T cells

Connor A. Tsuchida,[1,2,30] Nadav Brandes,[3,30] Raymund Bueno,[3,30,31] Marena Trinidad,[2] Thomas Mazumder,[3] Bingfei Yu,[4,5,32,33] Byungjin Hwang,[3,34] Christopher Chang,[6,7,8,9,10] Jamin Liu,[1,11,35] Yang Sun,[3] Caitlin R. Hopkins,[12,13,14,15,16] Kevin R. Parker,[4,36] Yanyan Qi,[17] Laura Hofman,[2,18] Ansuman T. Satpathy,[5,10,17] Edward A. Stadtmauer,[12,19] Jamie H.D. Cate,[20,21,22] Justin Eyquem,[8,9,10] Joseph A. Fraietta,[12,13,14,15,16] Carl H. June,[12,13,14,15] Howard Y. Chang,[4,5,23] Chun Jimmie Ye,[1,3,9,10,24,25,26,27,*] and Jennifer A. Doudna[1,2,10,20,21,22,28,29,37,*]

"[In a comparison of] the results from our laboratory experiments (where substantial chromosome loss was detected) and our clinical trial (where we did not observe chromosome loss above background levels), there were multiple technical differences in the parameters used for chromosome loss estimation.

We tried to account for these differences by downsampling the CROP-seq screen dataset so that its parameters were similar to those of the clinical trial dataset, which was sparser. Even upon downsampling, our estimations of chromosome loss in the CROP-seq screen were comparable to the original complete dataset.

This supports the conclusion that biological rather than technical reasons explain the dramatic difference in chromosome loss estimation."

Tsuchida et al *Cell* (2023) 186: 4856-4582

Key conclusion

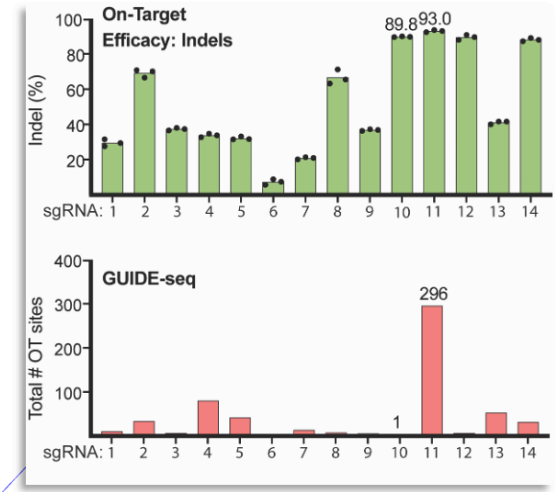
The presence in a human genome of a perfect sequence match, or partial match, to a gRNA spacer that Cas9 can carry is of questionable utility in determining the potency or the outcome spectrum of genome editing using that Cas9/gRNA in a living human cell.

Key conclusion

The presence in a human genome of a perfect sequence match, or partial match, to a gRNA spacer that Cas9 can carry is of **questionable utility** in determining the potency or the outcome spectrum of genome editing using that Cas9/gRNA in a living human cell.

Context is critical in determining the outcomes of genome editing in a primary human cell:

- What Cas9 was used? In what form?
- What gRNA?
- What chemical composition of both?
- Targeted to what sequence?
- Delivered how and at what amount of each?
- Into what kind of cells?
- How were the cells handled before and after genome editing?
- What were the functional consequences of editing on the cells in the near- and long-term?



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‡]

Introduction of Double-Strand Breaks into the Genome of Mouse Cells by Expression of a Rare-Cutting Endonuclease

PHILIPPE ROUET, FATIMA SMIH, AND MARIA JASIN*

Cell Biology and Genetics Program, Sloan-Kettering Institute and Cornell University Graduate School of Medical Sciences, New York, New York 10021

DSB drives edits

Genome editing native genes

Genome editing with Cas9

1994

2001-2005

2012

2023

Maria Jasin | Dana Carroll, Matthew Porteus, David Baltimore, Sangamo, others | Jennifer Doudna, Emmanuelle Charpentier | Lazzarotto et al *Nature Biotechnology* (2022) 38: 1317-1327

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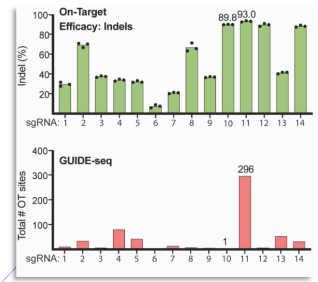

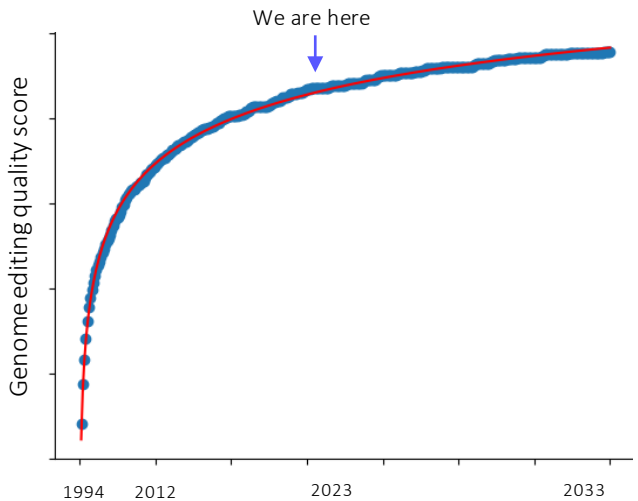

Introduction of Double-Strand Breaks into the Genome of Mouse Cells by Expression of a Rare-Cutting Endonuclease

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DSB drives edits

Genome editing native genes

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

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Genome editing with Cas9

1994 2001-2005 2012 2023 2033

Maria Jasin | Dana Carroll, Matthew Porteus, David Baltimore, Sangamo, others | Jennifer Doudna, Emmanuelle Charpentier | Lazzarotto et al *Nature Biotechnology* (2022) 38: 1317-1327