# 2020 CRISPR Year in Review

Science Circle
January $2^{nd}$ 2021

Stephen Gasior, Ph.D.
a.k.a. Stephen Xootfly

# 2020 CRISPR

Researcher at Corteva some of whose research is presented here. Not representing the company's positions.

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

# 2020 CRISPR

Nobel Prize—History to CRISPR

Technology Advances

Target Genes and Modified Organisms

chromosome rearrangements, clinical trials

# 2020 CRISPR

7 October 2020
The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2020 to

Emmanuelle Charpentier
Max Planck Unit for the Science of Pathogens, Berlin, Germany

Jennifer A. Doudna
University of California, Berkeley, USA

"for the development of a method for genome editing"

# CRISPR Timeline (Broad)

Discovery of CRISPR and its function
1993 - 2005 — Francisco Mojica, University of Alicante, Spain
(Pourcel et al, Bolotin et al) — France

Discovery of Cas9 and PAM
May, 2005 — Alexander Bolotin, French National Institute for Agricultural Research (INRA)

Hypothetical scheme of adaptive immunity
March, 2006 — Eugene Koonin, US National Center for Biotechnology Information, NIH

Experimental demonstration of adaptive immunity
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Spacer sequences are transcribed into guide RNAs
August, 2008 — John van der Oost, University of Wageningen, Netherlands

https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline

racecar stuff1 racecar stuff2 racecar stuff3 racecar

racecar racecar racecar racecar

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racecar virus1 racecar virus2 racecar virus3 racecar
racecar racecar racecar racecar

**Table 5.** Features of the sequences most similar to CRISPR spacers from *S. pyogenes*

| Spacer | Gene | Prophage[a] | Activity | Alignment[b] |
|---|---|---|---|---|
| 4-1 | *spyM3_1239* | 315.4 | Unknown | gctgtgacattgcgggatgtaatcaaagtaaaaa<br>gctgtgacattgcggaatgtaatcaaagcaaaaa |
| 4-2 | *spyM3_0941* | 315.2 | Capside protein | taaagcaaacctagcagaagcagaaaatgac<br>taaagcgaacctagtagaagcagaaaacgac |
| 4-3 | *spyM18_0741* | $\Phi_{spcC}$ | Methyltransferase | ctgatgtaattggtgattttcgtgatatgcttt<br>ctgatgtaattggtgattttcgtgatatgcctt |
| 7-1 | *spyM3_1215* | 315.4 | Endopeptidase | gcgctggttgatttcttcttgcgcttttt<br>gcgctggttgatttcttcttgcgcttttt |
| 7-2 | *speM* | $\Phi_{spcLM}$ | Exotoxin | tatatgaacataactcaatttgtaaaaaa<br>tatatgaacataactcaatttgtaaaaaa |
| 7-3 | *spyM18_0742* | $\Phi_{spcC}$ | Methyltransferase | aggaatatccgcaataattaattgcgctct<br>aggaatatccgcaataattaattgcgctct |
| 7-4 | *hylP* | 315.3 | Hyaluronidase | agtgccgaggaaaaattaggtgcgcttggc<br>agtgccgaggaaaaattaggtgcgcttggc |
| 7-5 | *spyM3_1347* | 315.5 | Unknown | aaatttgtttagcaggtaaaccgtgcttt<br>aaatttgtttagcaggtaaaccgtgcttt |

[a]Prophages 315.2-5 are integrated into *S. pyogenes* MGAS315. $\Phi_{speC}$ and $\Phi_{speLM}$ are integrated into *S. pyogenes* MGAS8232.
[b]CRISPR-spacer sequence (top line) and best-match homologous sequence (bottom line).

About 65% of the spacer homologs encountered correspond to bacteriophages or conjugative plasmids

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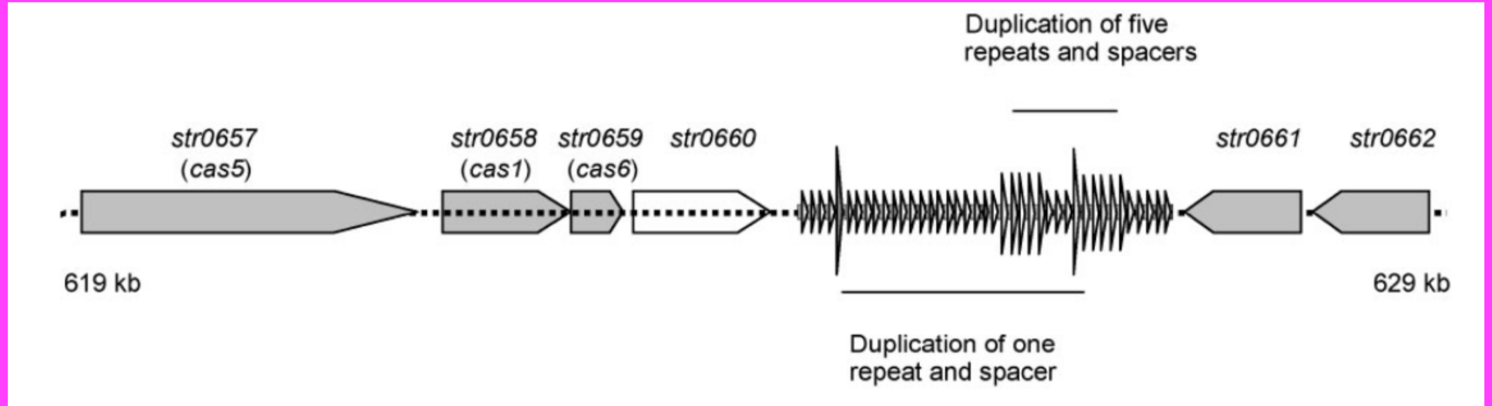


**Fig. 1.** The CRISPR locus in the *S. thermophilus* strain CNRZ1066. Repeats and spacers are shown as grey boxes and triangles, respectively, the duplicated spacers are shown as larger triangles, ORFs are represented as arrows (shaded for ORFs that have homologues in other genomes) and their designation in the *S. thermophilus* genome is given above the arrows. The numbers indicate the distance from the replication origin (in kb).

The Cas5 family groups large proteins (>1100 aa) that carry an HNH motif present in various nucleases, including colicin E9, which causes cell death by **introducing double- stranded breaks into DNA**, and a number of restriction enzymes

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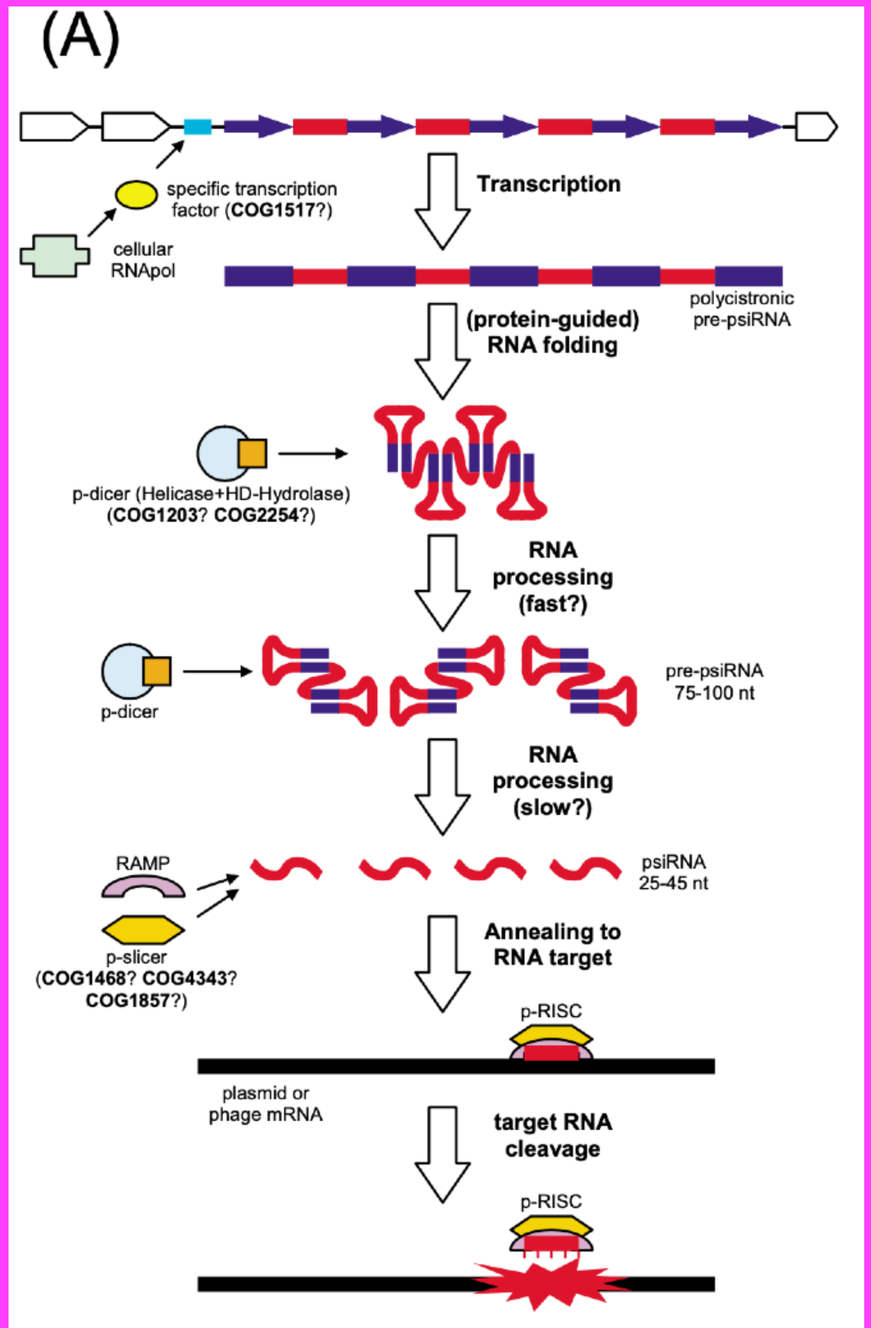


We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences. Removal or addition of particular spacers modified the phage-resistance phenotype of the cell. Thus, CRISPR, together with associated cas genes, provided resistance against phages, and resistance specificity is determined by spacer-phage sequence similarity. Danisco

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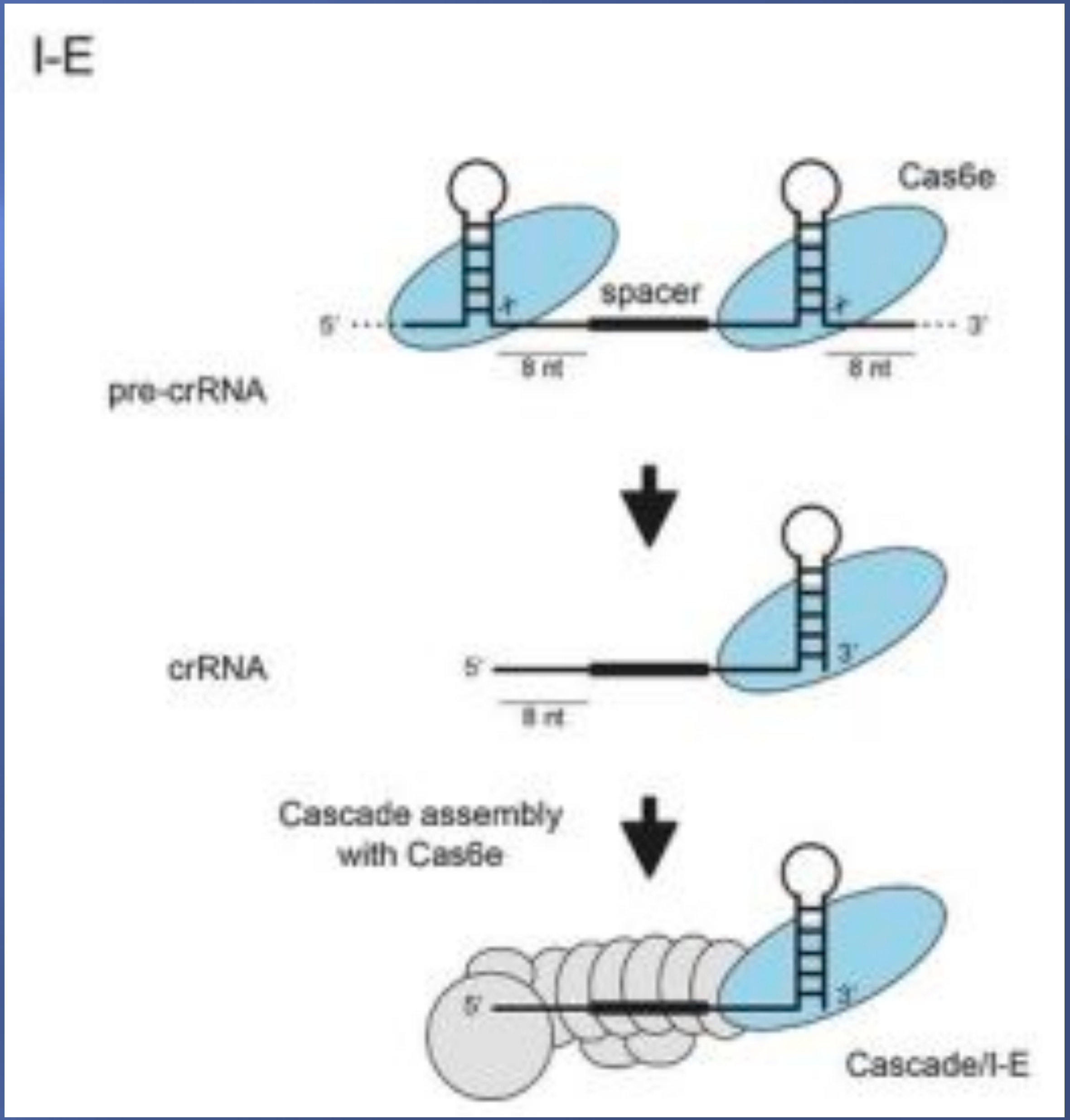

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**CRISPR acts on DNA targets**
**December, 2008 — Luciano Marraffini and Erik Sontheimer, Northwestern University, Illinois**

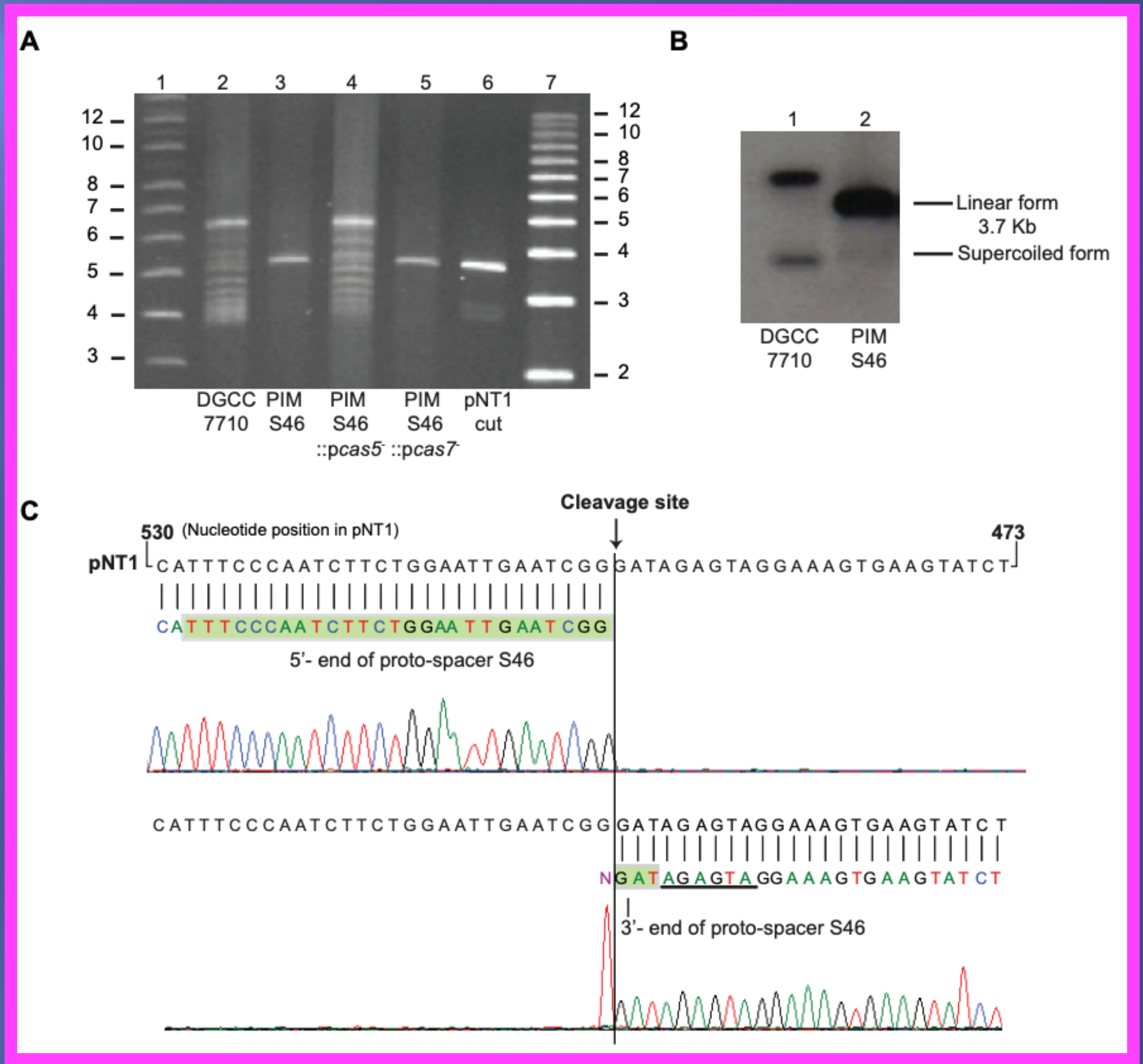
Cas9 cleaves target DNA
December, 2010 — Sylvain Moineau, University of Laval, Quebec City, Canada

Discovery of tracrRNA for Cas9 system
March, 2011 — Emmanuelle Charpentier, Umea University, Sweden and University of Vienna, Austria

CRISPR systems can function heterologously in other species
July, 2011 — Virginijus Siksnys, Vilnius University, Lithuania

Biochemical characterization of Cas9-mediated cleavage
September, 2012 — Virginijus Siksnys, Vilnius University, Lithuania

A clinical isolate of *Staphylococcus epidermidis* harbors a CRISPR spacer that matches the nickase gene present in nearly all staphylococcal conjugative plasmids. Here we show that CRISPR interference prevents conjugation and plasmid transformation in *S. epidermidis*. …indicates that the interference machinery targets DNA directly.



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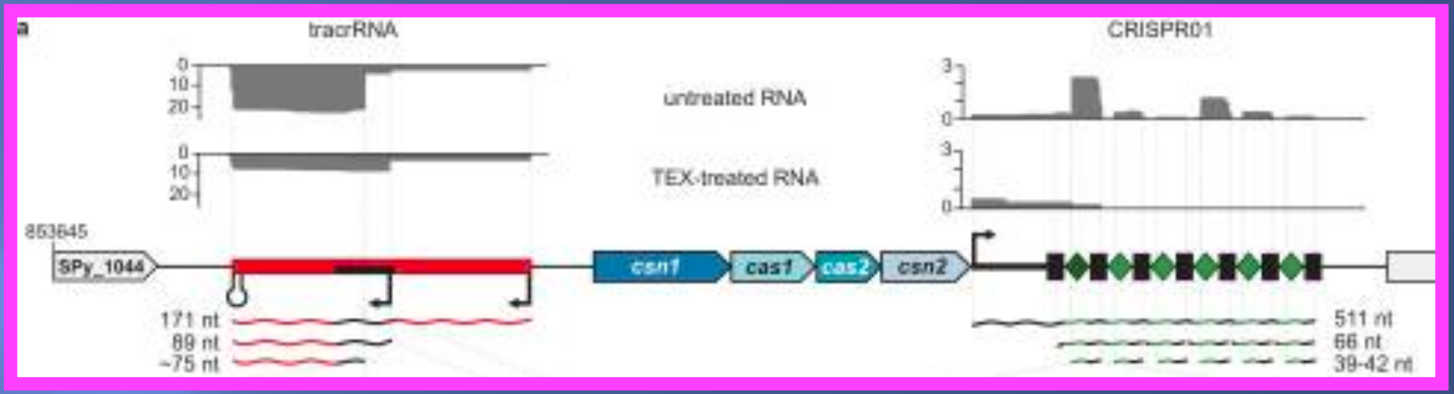


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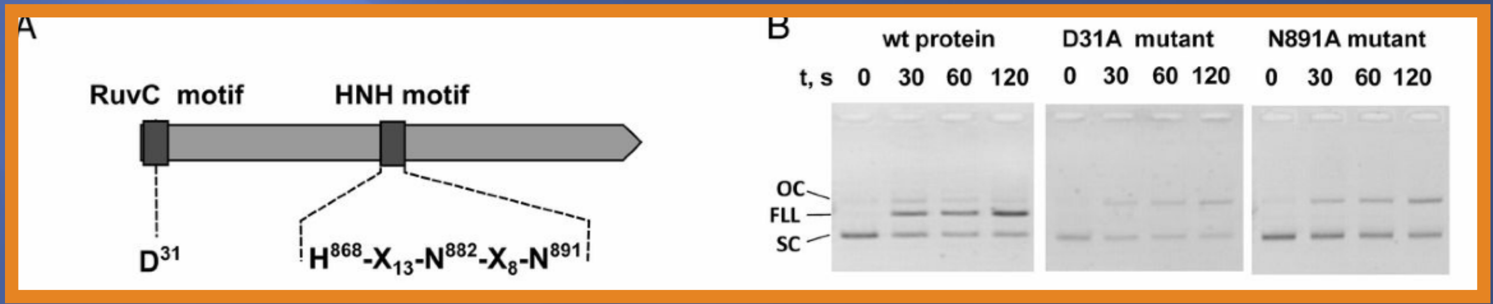


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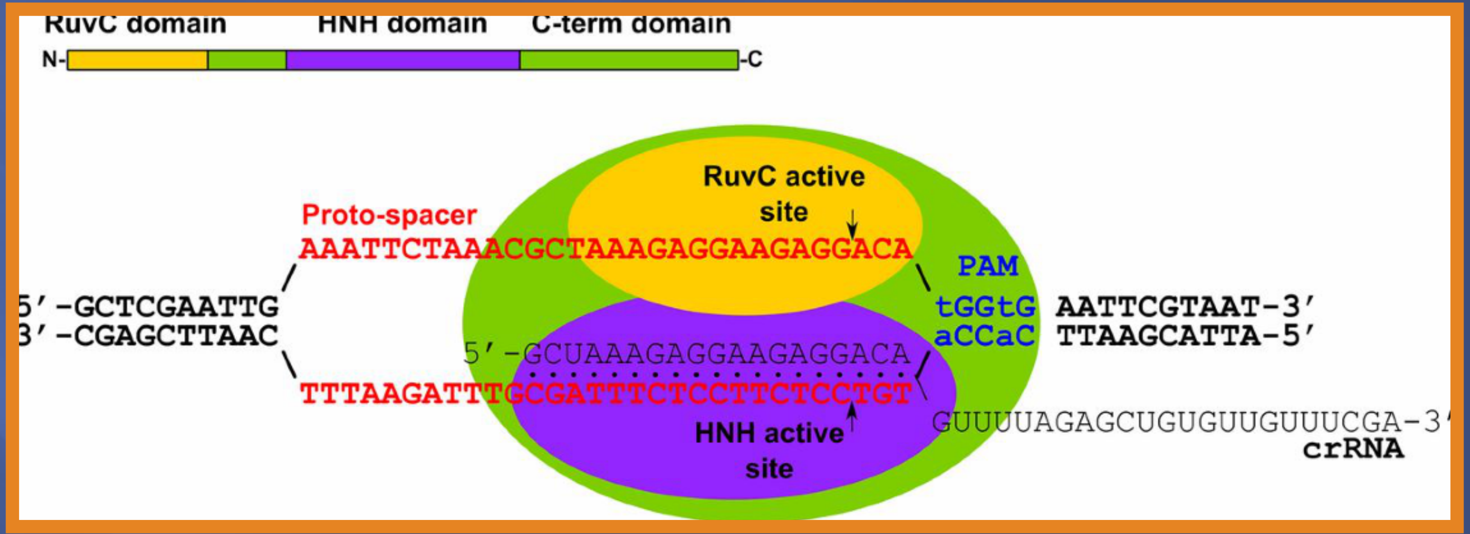


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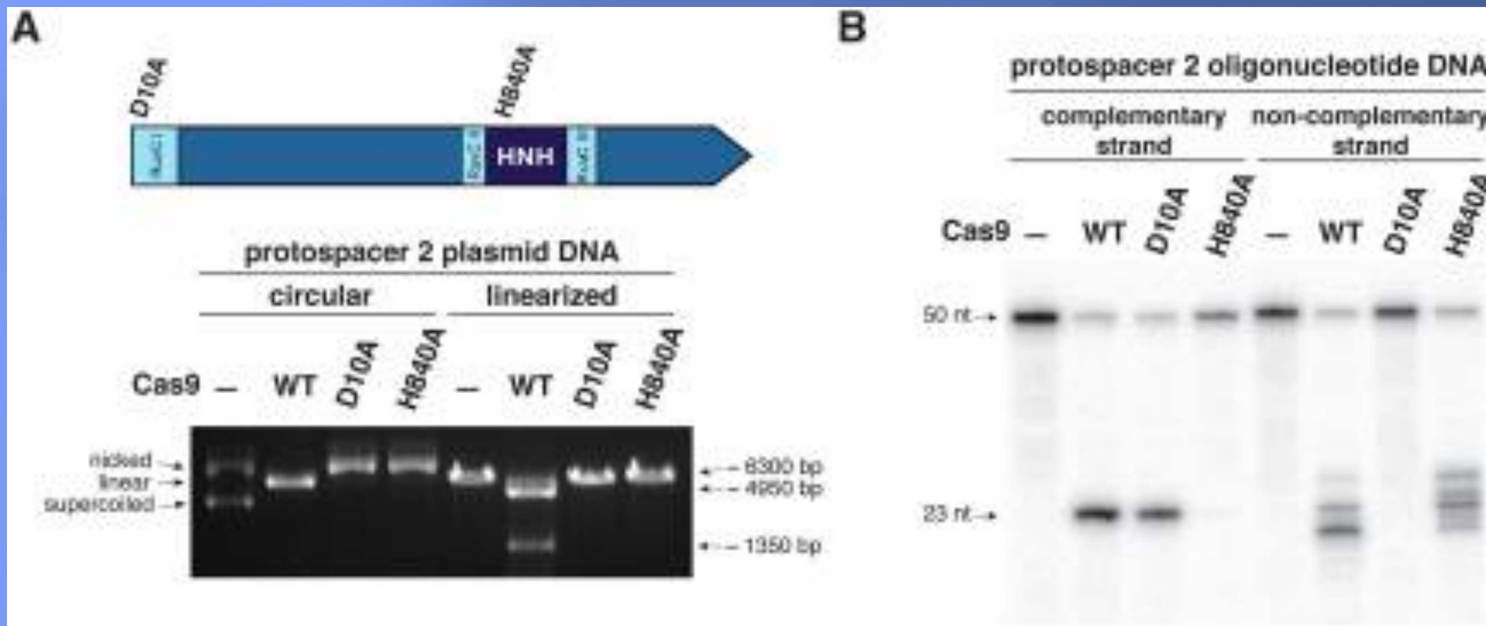
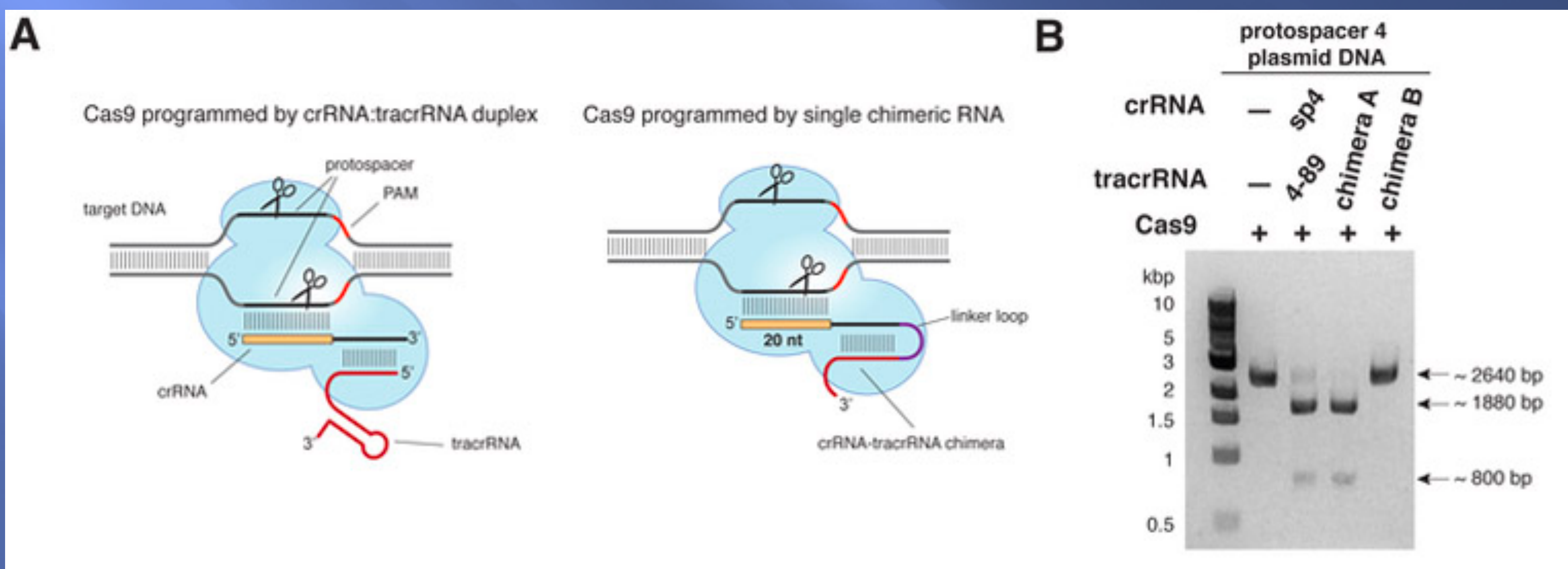
June, 2012 – Charpentier and Jennifer Doudna, University of California, Berkeley



# CRISPR Nobel

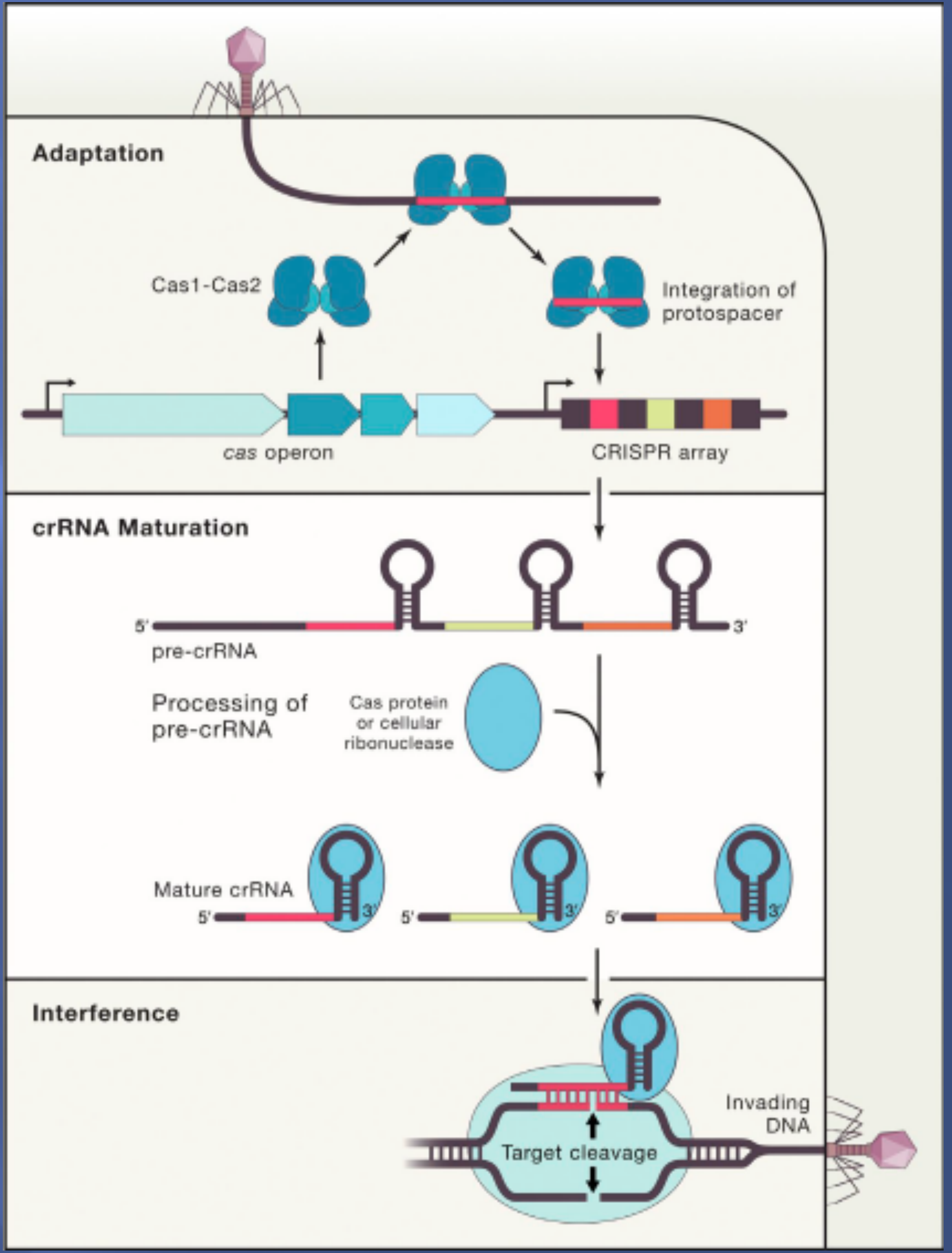
A weird thing in bacteria DNA

characterization of bacterial "immunity"

molecular and biochemical characterization

reprogramming for versatility

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES



# 1995 pre-CRISPR

## Genome Engineering – DSB connection

5012–5019 *Nucleic Acids Research, 1995, Vol. 23, No. 24*

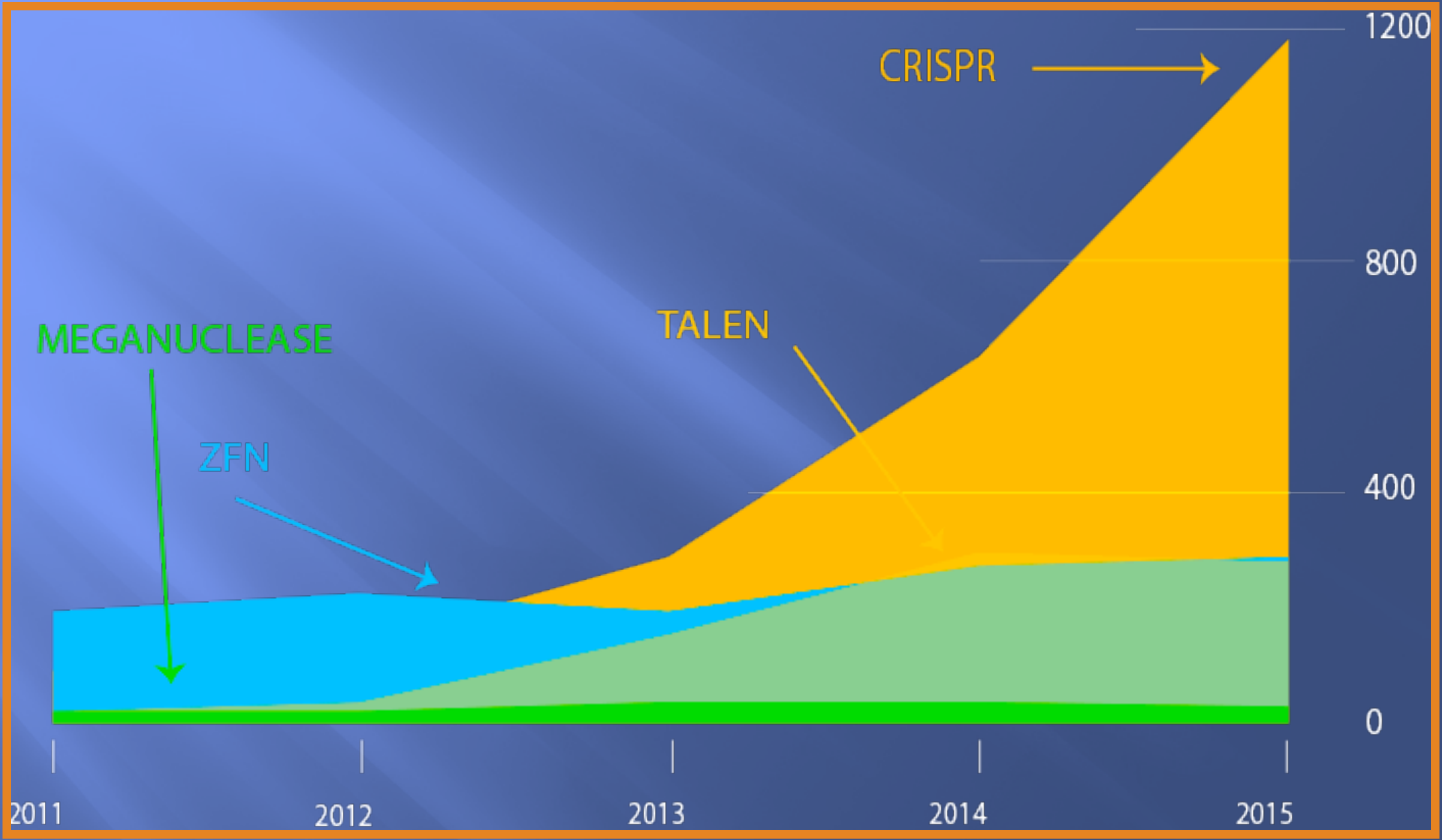
**Double-strand breaks at the target locus stimulate gene targeting in embryonic stem cells**

**Fatima Smih, Philippe Rouet, Peter J. Romanienko[1] and Maria Jasin***

Cell Biology and Genetics Program and [1]Molecular Biology Program, Sloan-Kettering Institute and Cornell University Graduate School of Medical Sciences, New York, NY 10021, USA

Received September 14, 1995; Accepted November 6, 1995

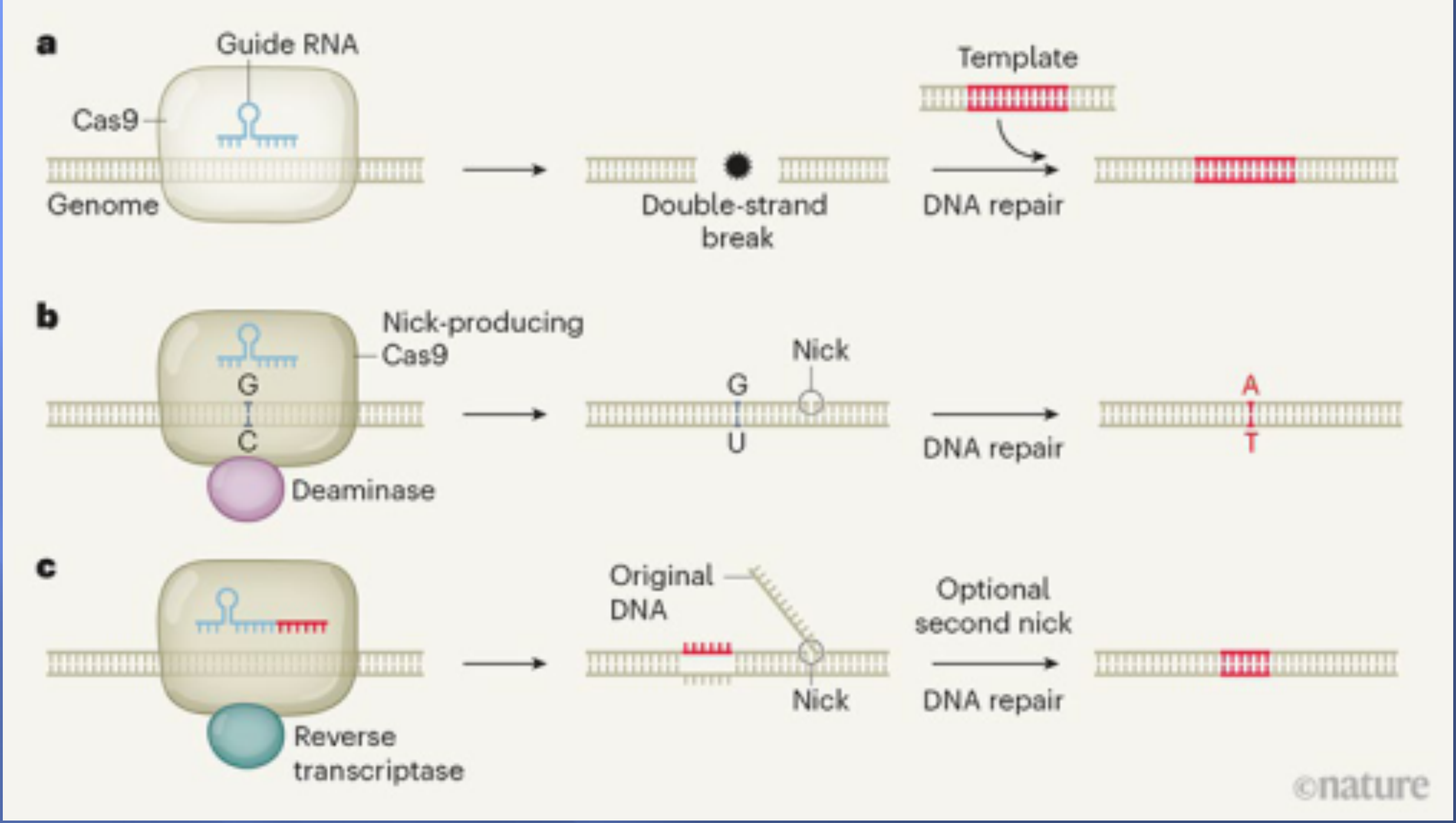
# CRISPR Citation Explosion




https://www.elsevier.com/research-intelligence/campaigns/crispr

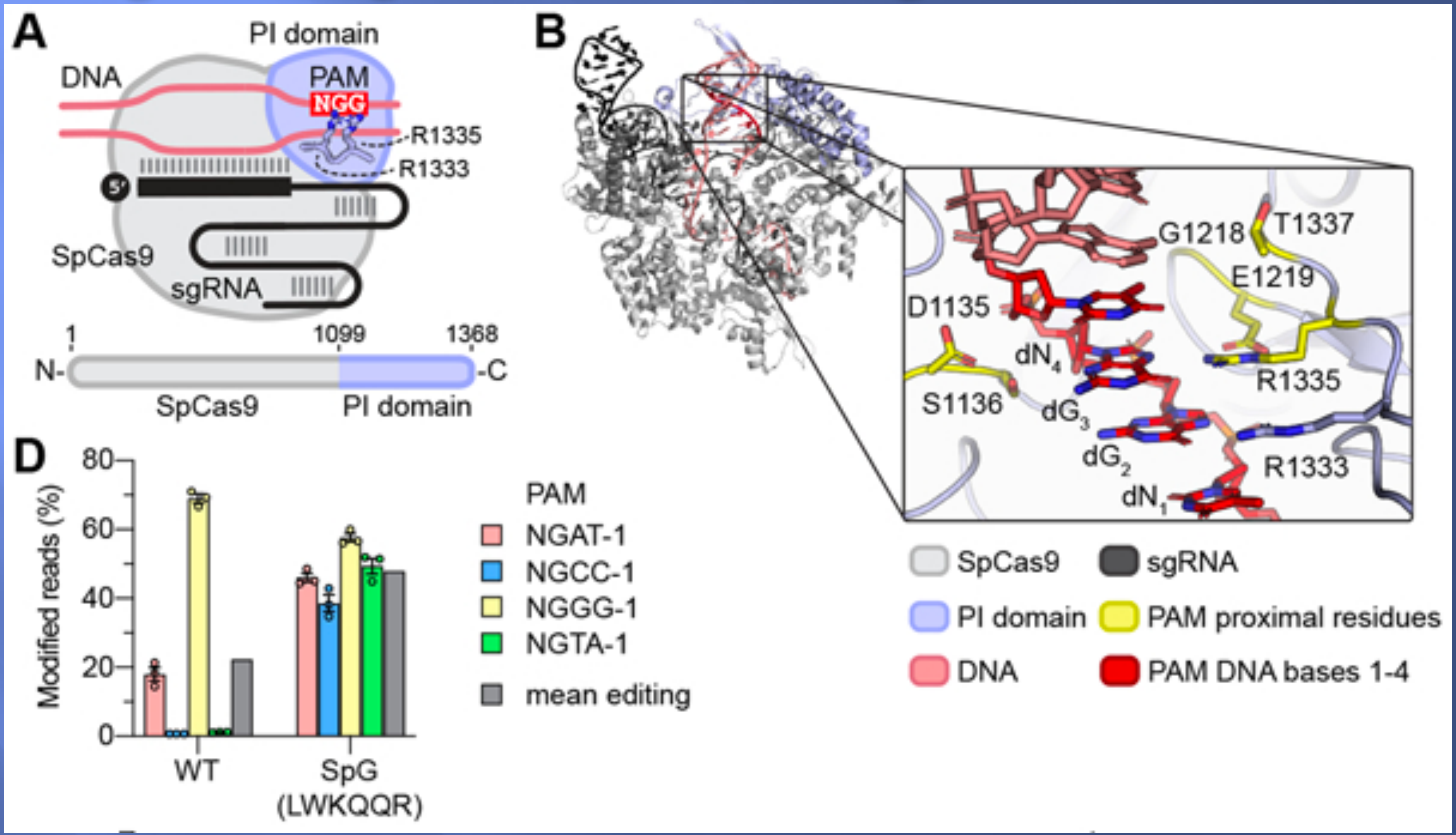
# CRISPR Nobel

a platform for engineering for biotechnology



# Technology Advances

## Engineering Cas9 overcoming PAM limitation




Walton, Russell T., et al. "Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants." Science 368.6488 (2020): 290-296.

# Target Genes and Modified Organisms

Sickle cell disease and β-thalassemia

edit stem cells to reactivate a fetal version of haemoglobin (Hbf) – CTX001 by Vertex Pharmaceuticals and CRISPR Therapeutics,

Promising clinical data for treatment

Cancer Immunotherapy

CRISPR inactivate PD1 which is a 'safety switch'

Passing safety trials

About Leber Congenital Amaurosis (LCA)

-inherited retinal degenerative disorders

-LCA10 caused by mutations in the CEP290 gene

-BRILLIANCE Trial – first direct injection of CRISPR

# Target Genes and Modified Organisms

## Rearranging Chromosomes

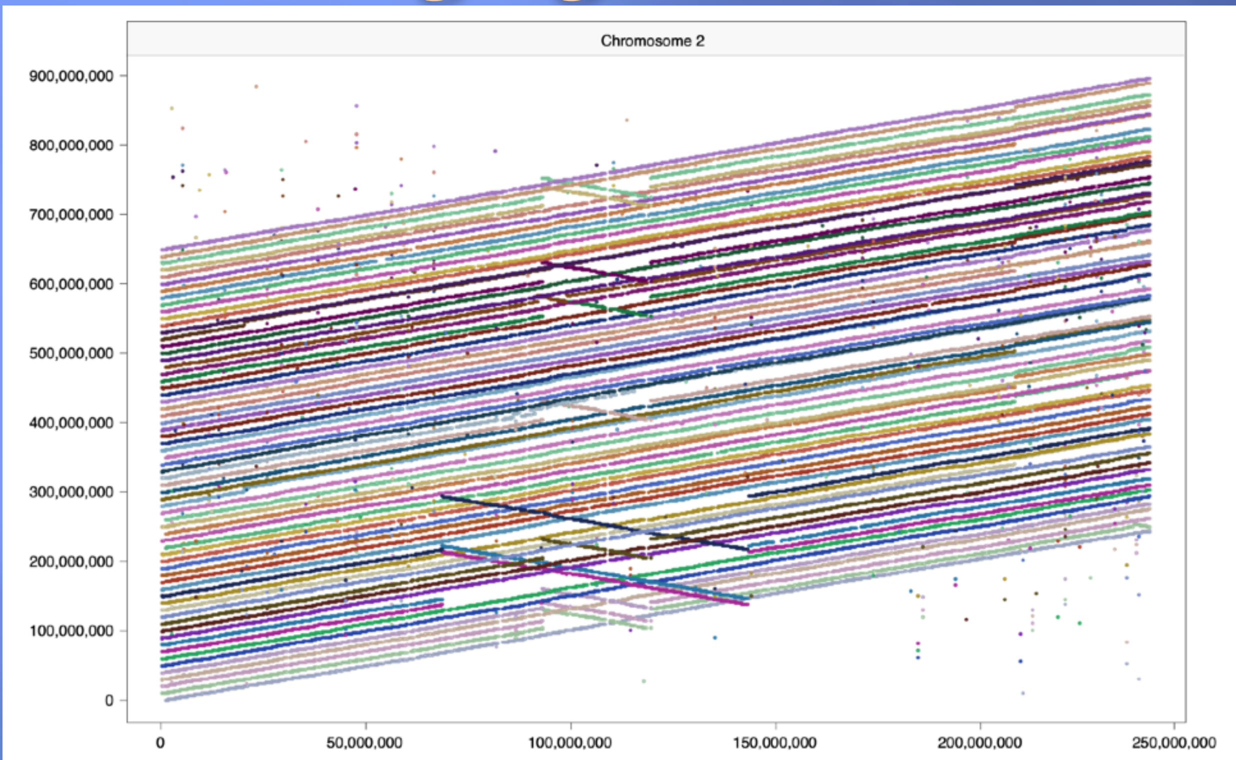



**Fig. 1 | Chromosome 2 pan-genome assembly comparison in 66 maize lines.** From this visualization scheme, several lines exhibit pericentric inversions relative to B73. Nine out of 66 lines contain a 26.7-Mb inversion, while three lines, including PH1V5T, exhibit a 75.5-Mb inversion.

Schwartz, Chris, et al. "CRISPR–Cas9-mediated 75.5-Mb inversion in maize." *Nature Plants* 6.12 (2020): 1427-1431.

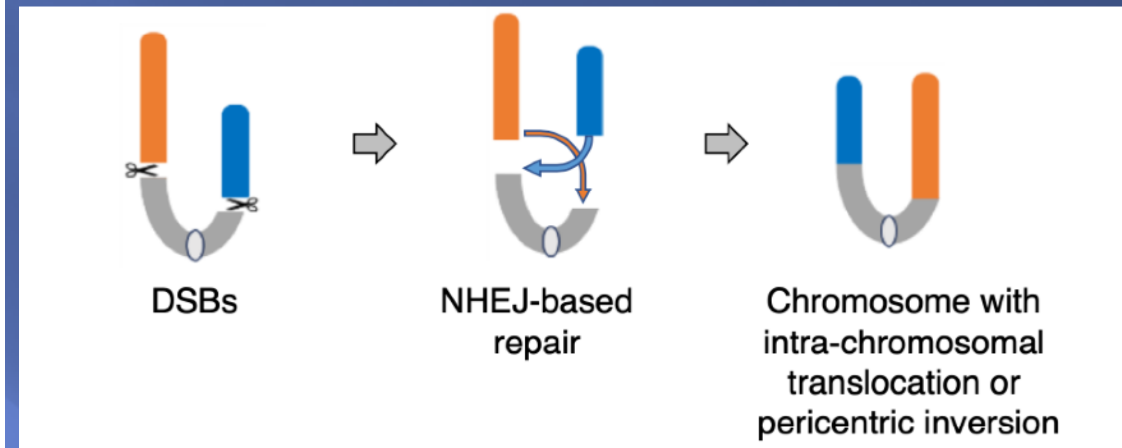
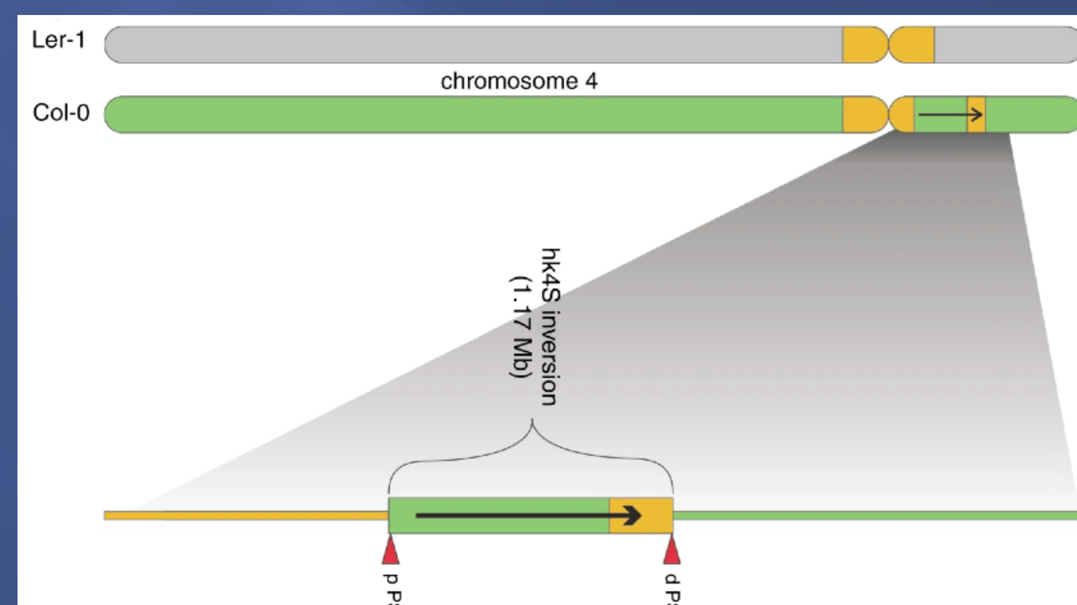



**Fig. 2 | Schematic illustration of the hypothetical mechanism of pericentric inversion.**

Schmidt, Carla, et al. "Changing local recombination patterns in Arabidopsis by CRISPR/Cas mediated chromosome engineering." *Nature communications* 11.1 (2020): 1-8.



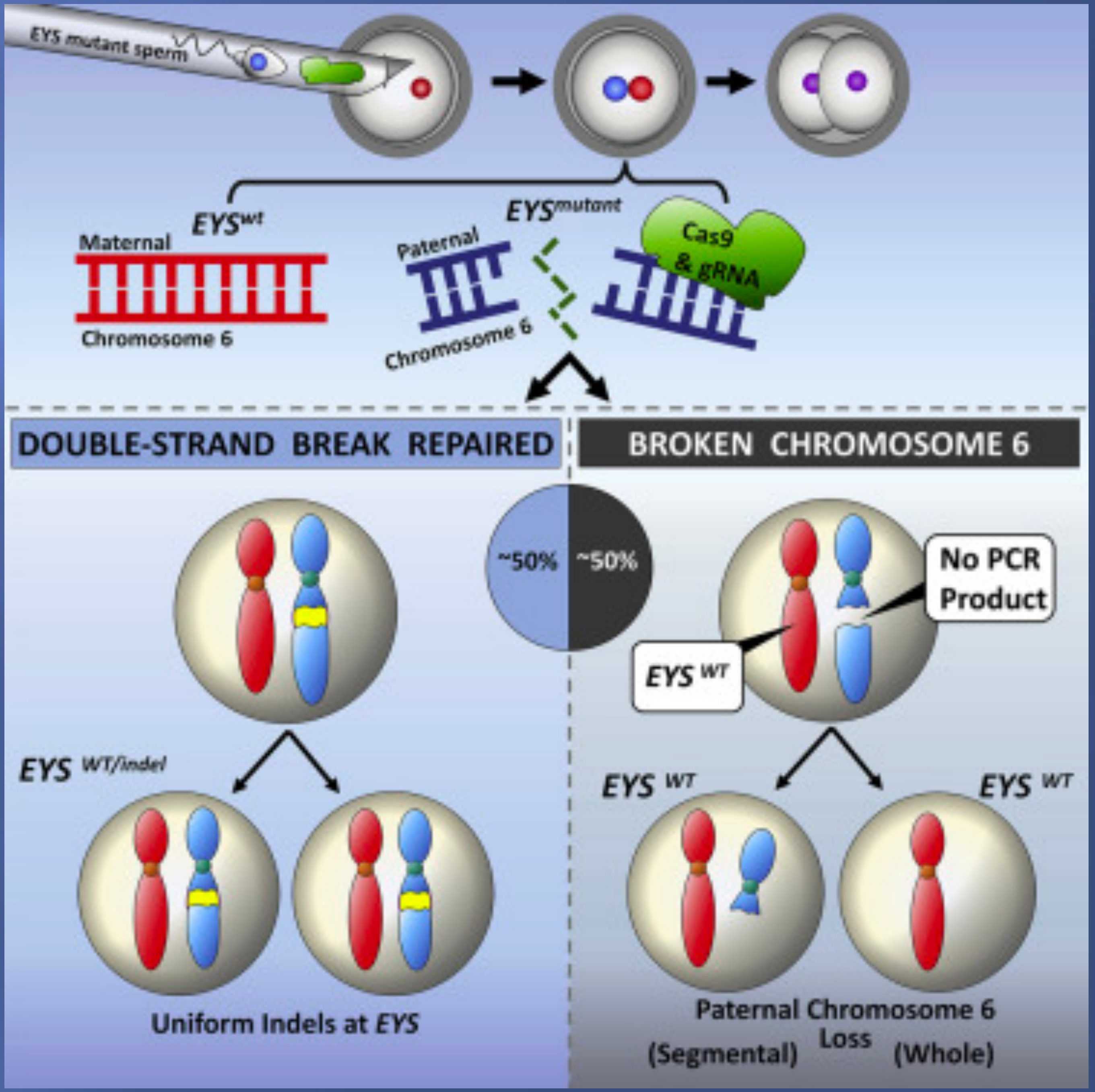
# Dangers of Gene Editing

## chromosome rearrangements and deletions in human embryos

Zuccaro, Michael V., et al. "Allele-specific chromosome removal after Cas9 cleavage in human embryos." *Cell* (2020).

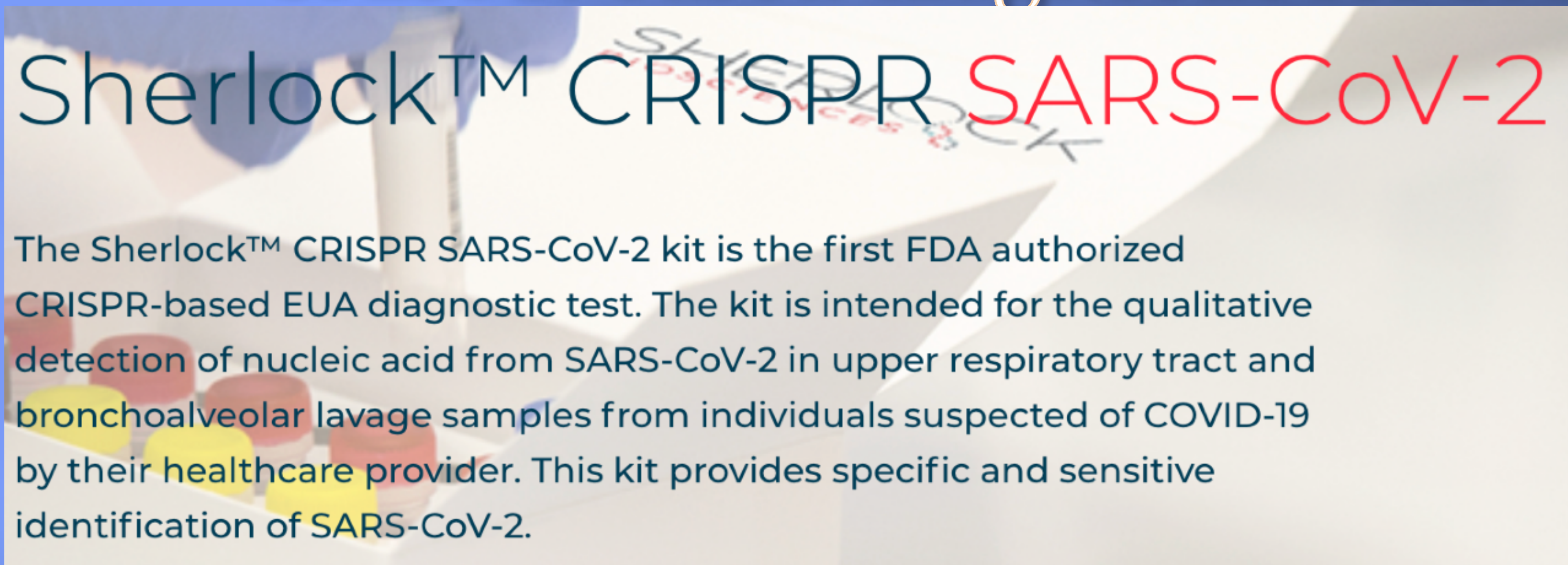
Mitalipov, Shoukhrat. "Frequent gene conversion in human embryos induced by double strand breaks." *bioRxiv* (2020).

Alanis-Lobato, Gregorio, et al. "Frequent loss-of-heterozygosity in CRISPR-Cas9-edited early human embryos." *bioRxiv* (2020).



# Cas Diagnostic

## CRISPR-based diagnostic for SARS-CoV-2

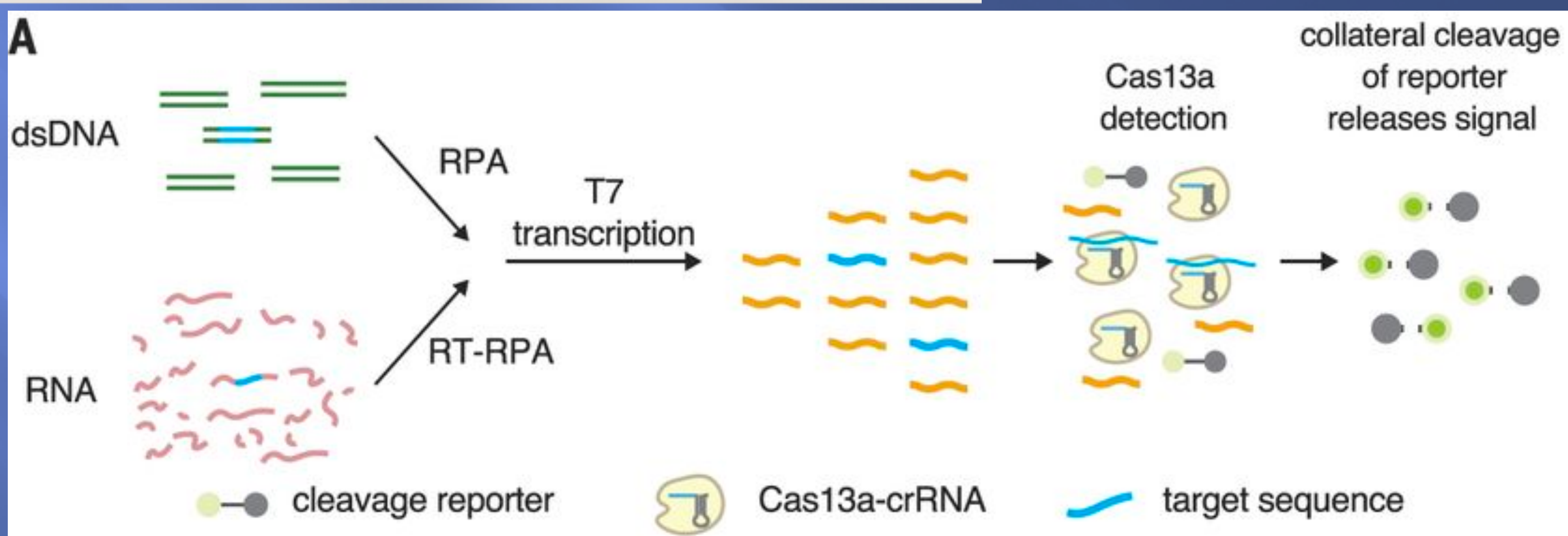



SHERLOCK BIOSCIENCES

https://sherlock.bio/crispr-sars-cov-2/

**Cas13**

RNA guided RNA endonuclease





Gootenberg, Jonathan S., et al. "Nucleic acid detection with CRISPR-Cas13a/C2c2." *Science* 356.6336 (2017): 438-442.

Ding, Xiong, et al. "Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay." *Nature communications* 11.1 (2020): 1-10.

# 2020 CRISPR Year in Review

Finite

Reminder for Fireside Chat
Monday 5 pm SLT

# References

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CRISPR TIMELINE
https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline
(CRISPR timeline articles are cited on that page)

Double-strand breaks at the target locus stimulate gene targeting in embryonic stem cells
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https://www.nature.com/articles/s41477-020-00817-6
Changing local recombination patterns in Arabidopsis by CRISPR/Cas mediated chromosome engineering
https://www.nature.com/articles/s41467-020-18277-z

# References

Chromosome rearrangement links
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https://europepmc.org/article/ppr/ppr178546
https://www.biorxiv.org/content/10.1101/2020.06.05.135913v2

Sherlock Biosciences
https://sherlock.bio/
Nucleic acid detection with CRISPR-Cas13a/C2c2
https://science.sciencemag.org/content/356/6336/438.long
Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay
https://www.nature.com/articles/s41467-020-18575-6