CRISPR-Cas in Plant Science:

From Mechanisms to Breeding Applications

Dr. ir. Jillis Grubben

Wageningen Research, Plant Breeding

November 12th, 2026



WAGENINGEN
UNIVERSITY & RESEARCH

Presentation contents

- My background
- CRISPR-Cas history
- CRISPR-Cas: What can we do with it?
- WURs own CRISPR enzyme: ThermoCas
- My perspective on CRISPR-Cas adoption in plant breeding

A brief introduction

Jillis Grubben

Researcher Plant Genome Editing

Plant Breeding, Wageningen Research

PhD, WUR (2025, cum laude)

CRISPR-Cas9-mediated targeted recombination in tomato

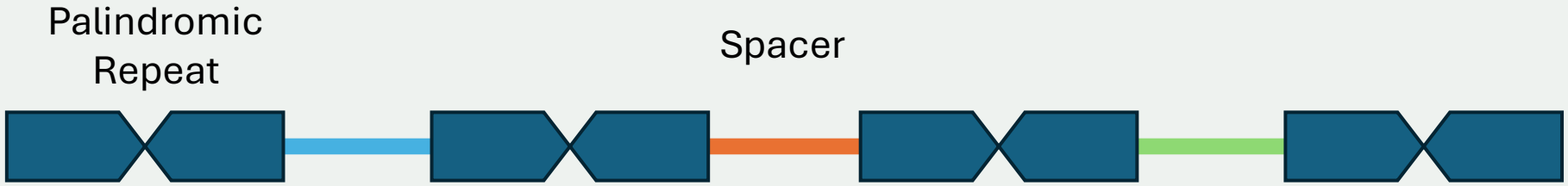
Current research

Optimising WUR's own CRISPR-ThermoCas enzyme for crop genome editing and targeted recombination

What is CRISPR-Cas? The discovery

WUR Microbiology – John van der Oost group

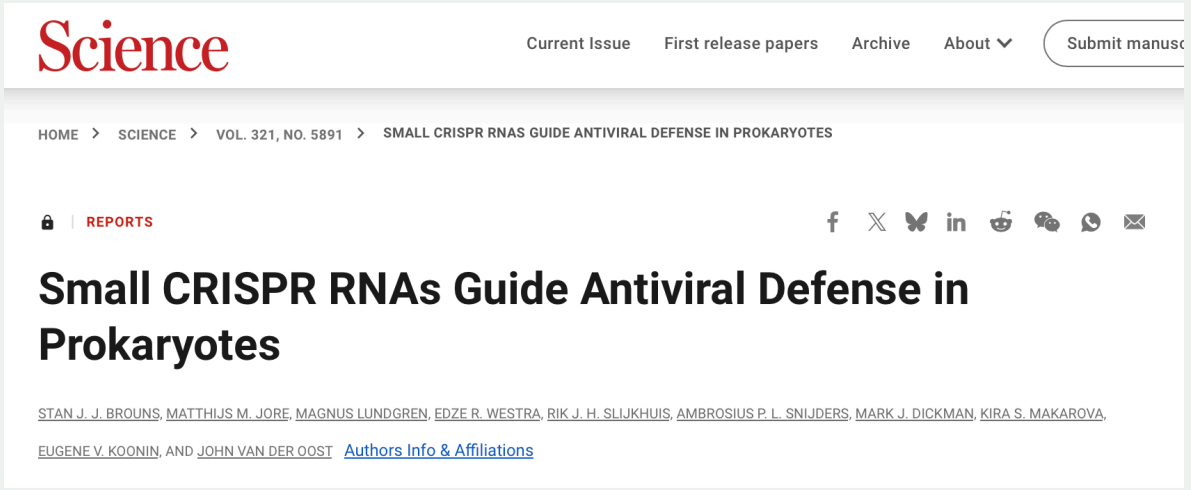
- Sequenced bacterial genomes
- They saw a repetitive, palindromic sequence (repeats)



- Interspaced with different DNA sequences (spacers)
- These were named: “Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)”
- Spacers appeared to be identical to viral sequences
- “Did bacteria use these to attack viruses?”

What is CRISPR-Cas? The discovery

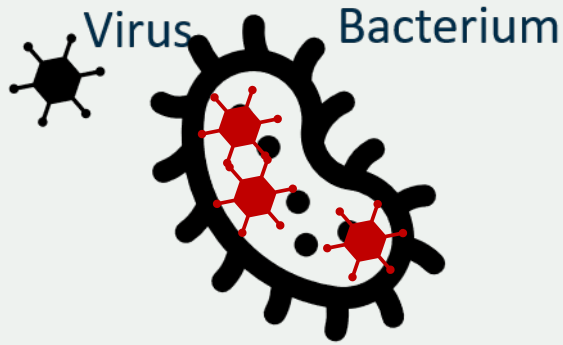
- “Did bacteria use these spacer sequences to attack viruses?”
- Answer: Yes
- Spacers are the bacterium's ‘memory’ used to attack viruses

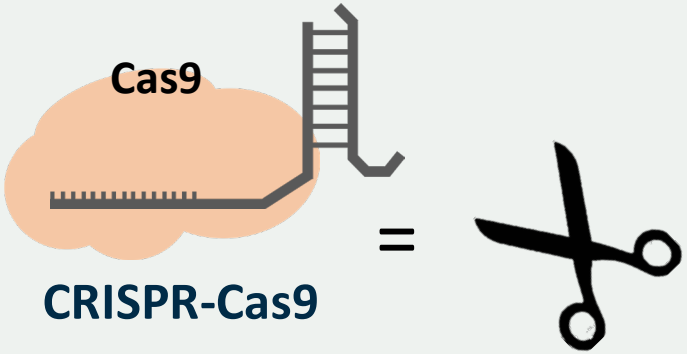


What is CRISPR-Cas? The discovery

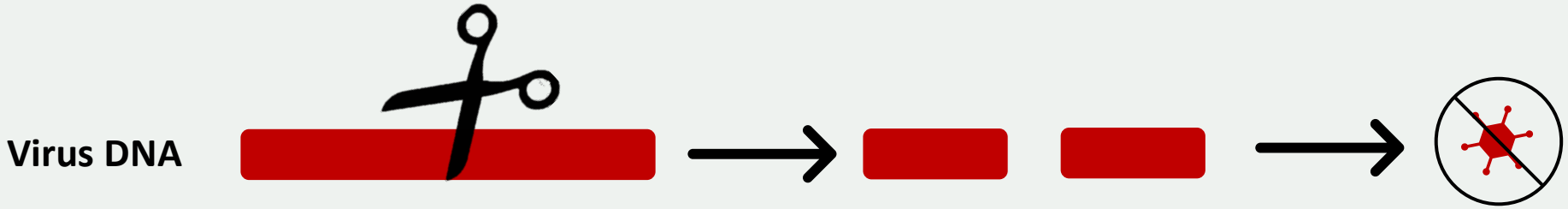
Mode of action:

1. Virus attacks bacterium
2. Bacterium grabs viral ‘memory’ sequence and forms a small RNA construct
3. This RNA construct forms a complex with the enzyme “Cas9”
4. CRISPR-Cas9; molecular pair of scissors





5. The complex scans the DNA in the bacterium, in search for the ‘memory’ sequence
6. Match: Cas9 cuts the viral DNA in two
7. Viral infection halted



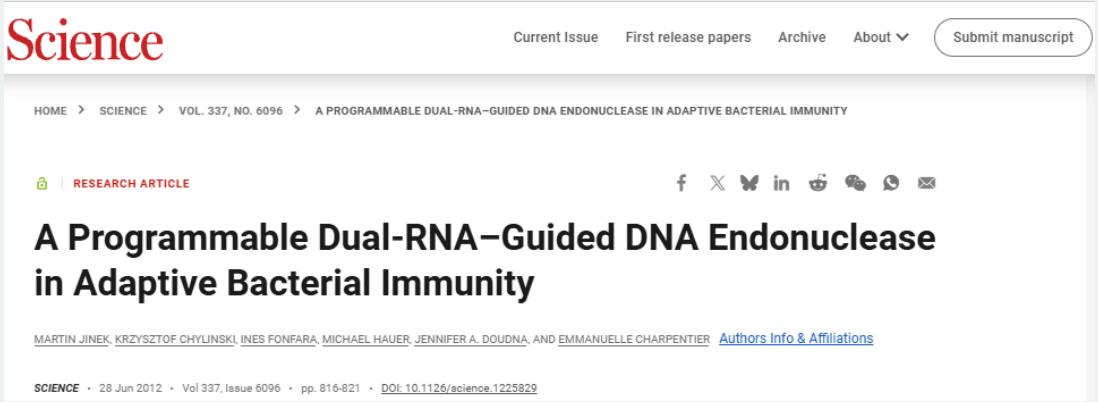
What is CRISPR-Cas? From defence mechanism to editing tool

- Others asked: “Can this system be tweaked and be used for targeted editing?”
- Answer: Yes

- Showed that we can modify the spacer sequence into a “guideRNA”
- Guiding of Cas9 to a sequence of choice

- Editing a target of choice

- CRISPR-Cas as we know it today



Nobel Prize in Chemistry 2020



Jennifer Doudna Emmanuelle Charpentier

January-February 2013:
1st reports on application of CRISPR-Cas in human cell lines

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

Functional elucidation of causal genetic variants and elements requires precise genome editing technologies. The type II prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats)/Cas adaptive immune system has been shown to facilitate RNA-guided site-specific DNA cleavage. We engineered two different type II CRISPR/Cas systems and demonstrate that Cas9 nucleases can be directed by short RNAs to induce precise cleavage at endogenous genomic loci in human and mouse cells. Cas9 can also be converted into a nicking enzyme to facilitate homology-directed repair with minimal mutagenic activity. Lastly, multiple guide sequences can be encoded into a single CRISPR array to enable simultaneous editing of several sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology.

Precise and efficient genome-targeting technologies are needed to enable systematic reverse engineering of causal genetic variations by allowing selective perturbation of individual genetic elements. Although genome-editing technologies such as designer zinc fingers (ZFs) (*1*–*4*), transcription activator–like effectors (TALEs) (*4*–*10*), and homing meganucleases (*11*) have begun to enable targeted genome modifications, there remains a need for new technologies that are scalable, affordable, and easy to engineer. Here, we report the development of a class of precision genome-engineering tools based on the RNA-guided Cas9 nuclease (*12*–*14*) from the type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system (*15*–*18*).

www.sciencemag.org SCIENCE VOL 339 15 FEBRUARY 2013

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,[1]* Luhan Yang,[1,3]* Kevin M. Esvelt,[2] John Aach,[1] Marc Guell,[1] James E. DiCarlo,[4] Julie E. Norville,[1] George M. Church[1,2]†

Bacteria and archaea have evolved adaptive immune defenses, termed clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, that use short RNA to direct degradation of foreign nucleic acids. Here, we engineer the type II bacterial CRISPR system to function with custom guide RNA (gRNA) in human cells. For the endogenous AAVS1 locus, we obtained targeting rates of 10 to 25% in 293T cells, 13 to 8% in K562 cells, and 2 to 4% in induced pluripotent stem cells. We show that this process relies on CRISPR components; is sequence-specific; and, upon simultaneous introduction of multiple gRNAs, can effect multiplex editing of target loci. We also compute a genome-wide resource of ~190 K unique gRNAs targeting ~40.5% of human exons. Our results establish an RNA-guided editing tool for facile, robust, and multiplexable human genome engineering.

Bacterial and archaeal clustered regularly interspaced short palindromic repeats (CRISPR) systems rely on CRISPR RNAs (crRNAs) in complex with CRISPR-associated (Cas) proteins to direct degradation of complementary sequences present within invading viral and plasmid DNA (*1*–*3*). A recent in vitro re-

[7]Computational and Syste
Koch Institute for Integral
Institute of Technology, C
ratory of Bacteriology, Th
Avenue, New York, NY 10
*These authors contribut
†To whom correspondenc
zhang@broadinstitute.o

nature biotechnology

Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease

Seung Woo Cho[1–3], Sojung Kim[1–3], Jong Min Kim[1,2] & Jin-Soo Kim[1,2]

We employ the CRISPR-Cas system of *Streptococcus pyogenes* as programmable RNA-guided endonucleases (RGENs) to cleave DNA in a targeted manner for genome editing in human cells. We show that complexes of the Cas9 protein and artificial chimeric RNAs efficiently cleave two genomic sites and induce indels with frequencies of up to 33%.

The clustered, regularly interspaced, short palindromic repeats (CRISPR)–CRISPR-associated protein (Cas) system provides prokary-

RFP and GFP sequences in human embryonic kidney (HEK) 293T cells (**Fig. 1c**). In this reporter, the GFP sequence is fused to the RFP sequence out-of-frame. GFP is expressed only when the target sequence is cleaved by site-specific nucleases, which causes small, frameshifting insertions or deletions around the target sequence by means of error-prone nonhomologous end joining repair of the double-strand breaks. We found that GFP-expressing cells were obtained at frequencies ranging from 5% to 7% in three independent experiments only when the cells were co-transfected with the *in vitro* transcribed chimeric RNA and a plasmid encoding Cas9, demonstrating that sgRNA:Cas9 can recognize and cleave the target DNA sequence in eukaryotic cells.

To test whether the RGENs could be used for targeted disruption of endogenous genes in human cells, we analyzed genomic DNA isolated from transfected cells using T7 endonuclease I (T7EI), an endonuclease that specifically cleaves heteroduplexes formed by the hybridization of wild-type and mutant DNA sequences[5]. We found that mutations were induced only when the cells were co-transfected with both Cas9 and

WAGENINGEN
UNIVERSITY & RESEARCH

August 2013:
1st reports on application of CRISPR-Cas in plants

Open
LETTER TO THE EDITOR

Cell Research (2013) 23:1229-1232.
© 2013 IBCB, SIBS, CAS All rights reserved 1001-0602/13
www.nature.com/cr

Efficient genome editing in plants using a CRISPR/Cas system

Cell Research (2013) **23**:1229-1232. doi:10.1038/cr.2013.114; published online 20 August 2013

Dear Editor,

In the past few years, the development of seq
specific DNA nucleases has progressed rapidly an
nucleases have shown their power in generating e

© 2013 Nature America, Inc. All rights reserved.

AUTHOR CONTRIBUTIONS
Q.Z. designed the experiments, supervised laboratory work, analyzed and interpreted data; Q.Z. and W.L. wrote the paper; W.L., F.T. and T.L. performed the experiments.

10. Tesson, L. *et al. Nat. Biotechnol.* **29**, 695–696 (2011).
11. Wu, H. & Zhang, Y. *Genes Dev.* **25**, 2436–2452 (2011).
12. Gu, T.P. *et al. Nature* **477**, 606–610 (2011).
13. Dawlaty, M.M. *et al. Dev. Cell* **24**, 310–323 (2013).

Targeted genome modification of crop plants using a CRISPR-Cas system

To the Editor:
Although genome editing technologies using zinc finger nucleases (ZFNs)[1] and transcription activator-like effector nucleases (TALENs)[2] can generate genome modifications, new technologies that are robust, affordable and easy to engineer are needed. Recent advances in the study of

show that customizable sgRNAs can direct Cas9 to induce sequence-specific genome modifications in the two most widely cultivated food crops, rice (*Oryza sativa*) and common wheat (*Triticum aestivum*).

We first codon-optimized *Streptococcus pyogenes Cas9* (*SpCas9*), attached nuclear localization signals (NLSs) at both ends

Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease

To the Editor:
Sustainable intensification of crop production is essential to ensure food demand is matched by supply as the human population continues to increase[1]. This will require high-yielding crop varieties that can be grown sustainably with fewer inputs on less land. Both plant breeding and genetic modification (GM)

nucleases (TALENs) can be engineered to create such breaks, but these systems require two different DNA binding proteins flanking a sequence of interest, each with a C-terminal FokI nuclease module. We report here that the bacterial clustered, regularly interspaced, short palindromic repeats (CRISPR) system, comprising a CRISPR-associated (Cas)9

What is CRISPR-Cas? The tool explained

CRISPR-Cas comes in different flavours:

- Double strand DNA cut (e.g. Cas9, Cas12a, ThermoCas, …)
- Targeting RNA instead of DNA (Cas13)
- DNA ‘shredder’ (deletion of up to 100.000 bp) (Cascade-Cas3)
- Etc ...

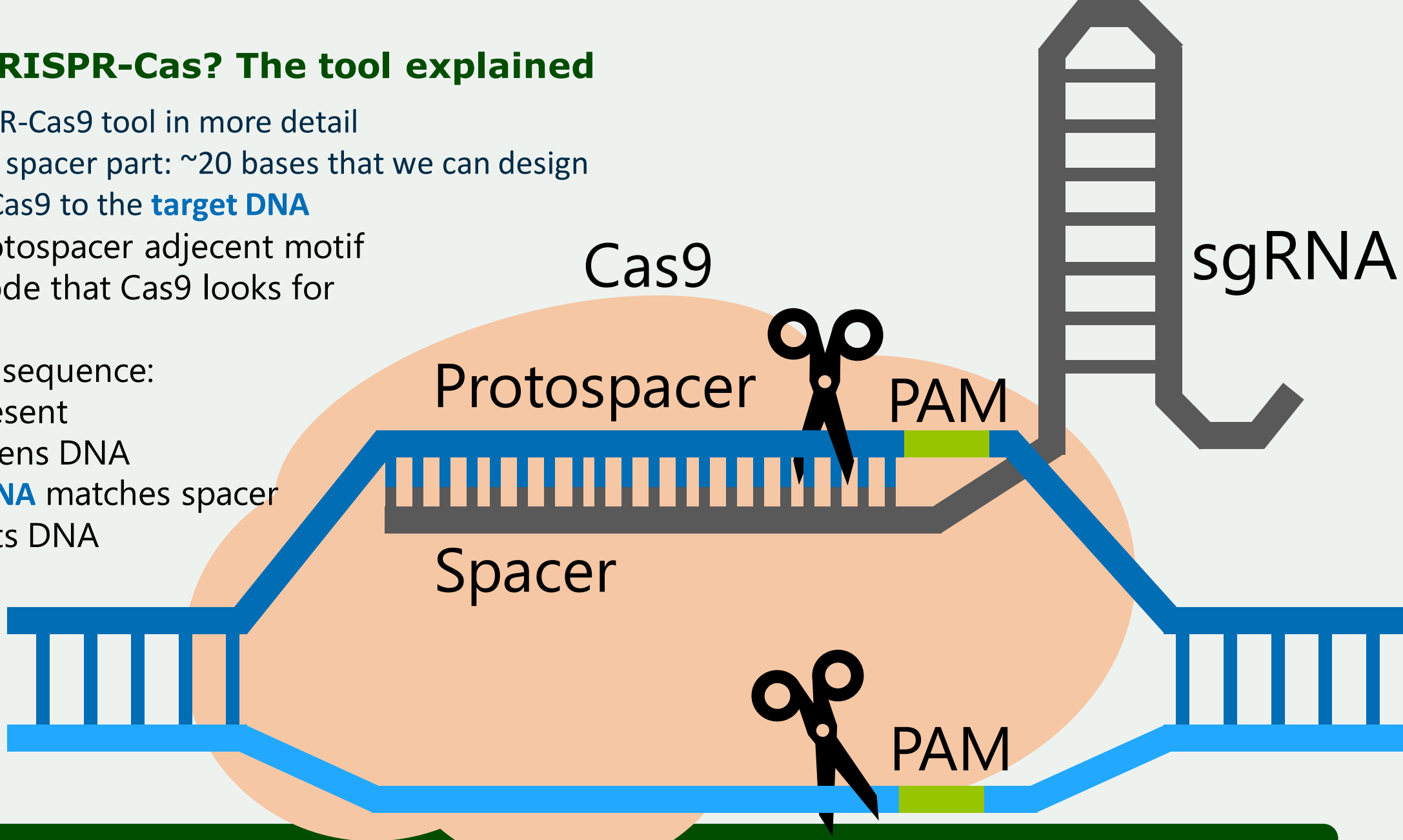
What does the CRISPR-Cas complex look like?

What is CRISPR-Cas? The tool explained

- The CRISPR-Cas9 tool in more detail
- guideRNA spacer part: ~20 bases that we can design
- It guides Cas9 to the **target DNA**
- **PAM:** Protospacer adjecent motif
- 3 base code that Cas9 looks for

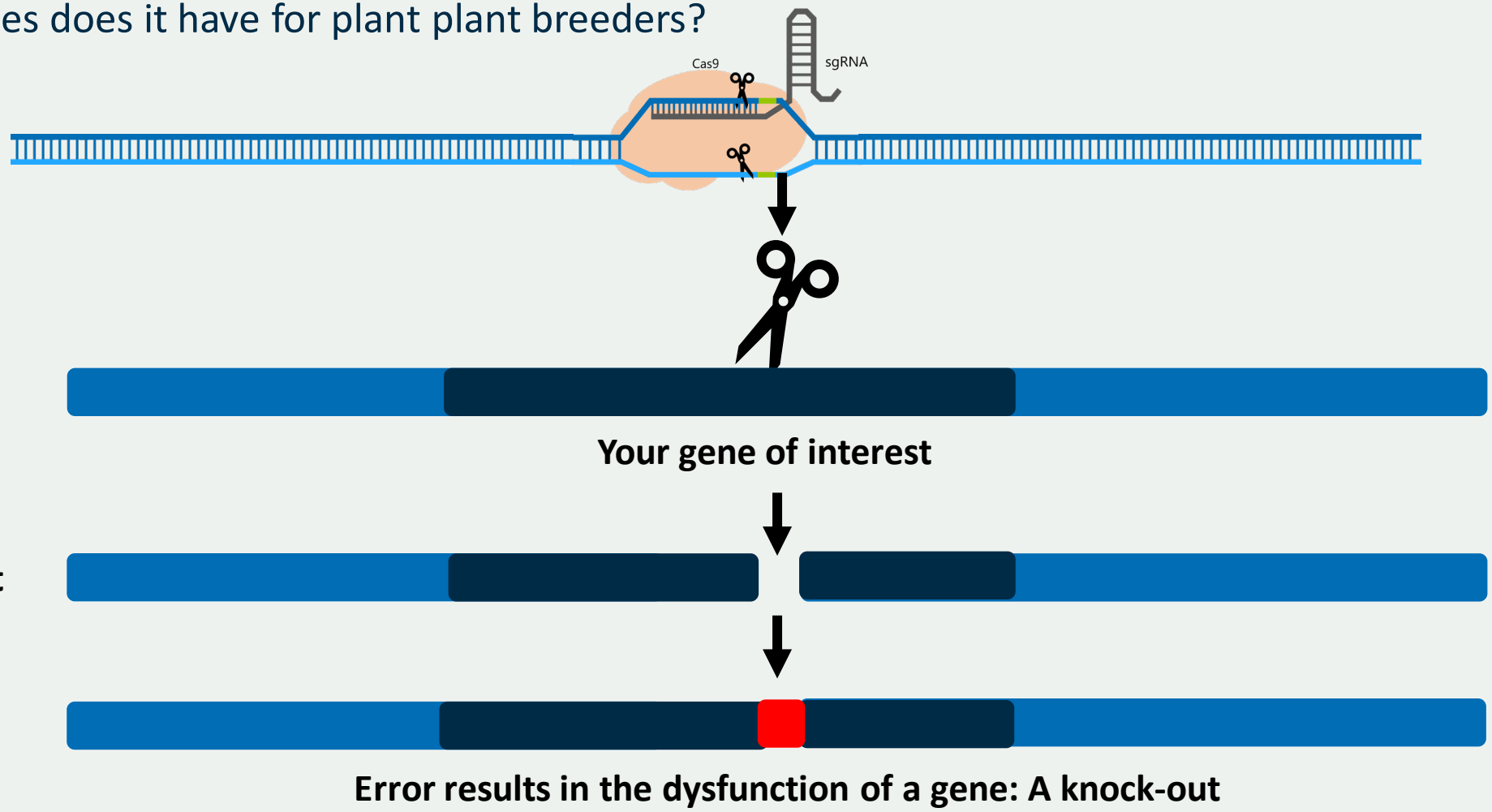
Functioning sequence:

1. PAM present
2. Cas9 opens DNA
3. **Target DNA** matches spacer
4. Cas9 cuts DNA
5. Done



What is CRISPR-Cas? What can we do with it?

- Zooming out: What uses does it have for plant plant breeders?



What is CRISPR-Cas? What can we do with it?

- Zooming out: What uses does it have for plant plant breeders?

DNA repaired with error



Gene no longer functional: Knock-out

By making a precise edit, we can:

1. Knock-out genes to find gene functions: “what function does this gene have?”
2. Knock-out of a susceptibility gene → pathogen loses host factor → much less infection
   Example: S-gene knockouts in potato[1, 2]
3. Edit a promoter instead of editing a gene[3] → tune gene expression (yield, fruit size)

1: Sun K., Wolters A.-M.A., Vossen J.H., Rouwet M.E., Loonen A.E.H.M., Jacobsen E., Visser R.G.F., Bai Y. (2016) Silencing of six susceptibility genes results in potato late blight resistance.
2: Karlsson M., Kieu N.P., Lenman M., Marttila S., Resjö S., Zahid M.A., Andreasson E. (2024) CRISPR/Cas9 genome editing of potato StDMR6-1 results in plants less affected by different stress conditions.
3: Rodríguez-Leal D., Lemmon Z.H., Man J., Bartlett M.E., Lippman Z.B. (2017) Engineering quantitative trait variation for crop improvement by genome editing.

What is CRISPR-Cas? What can we do with it?

Increasing complexity… use two guideRNAs

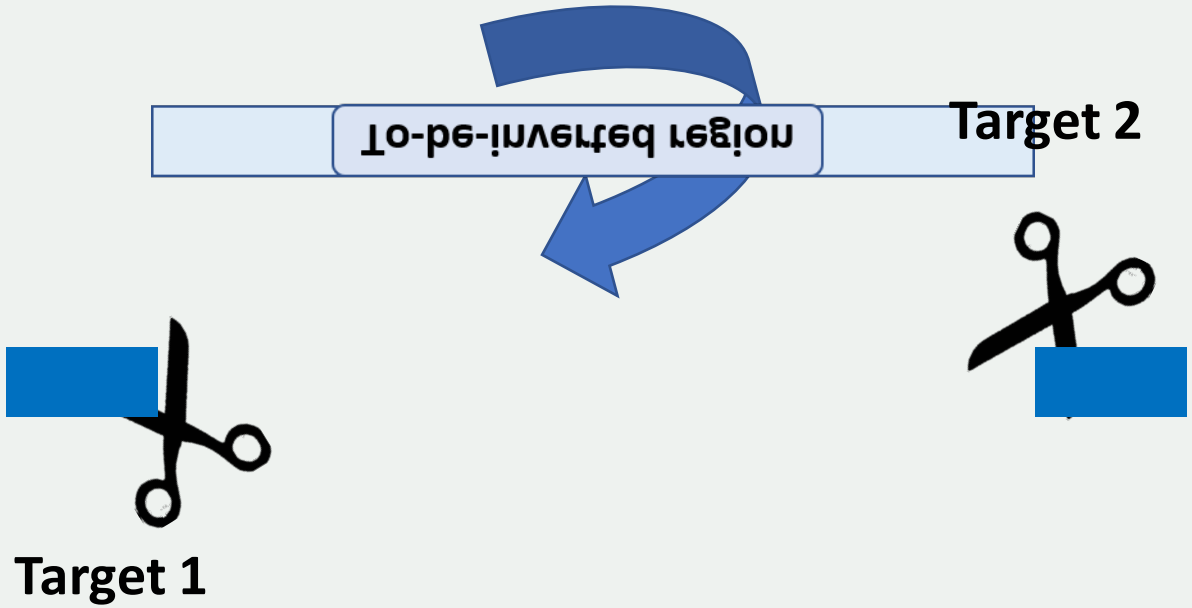
1. Cut
2. Excise



What is CRISPR-Cas? What can we do with it?

By making a precise edit, we can:

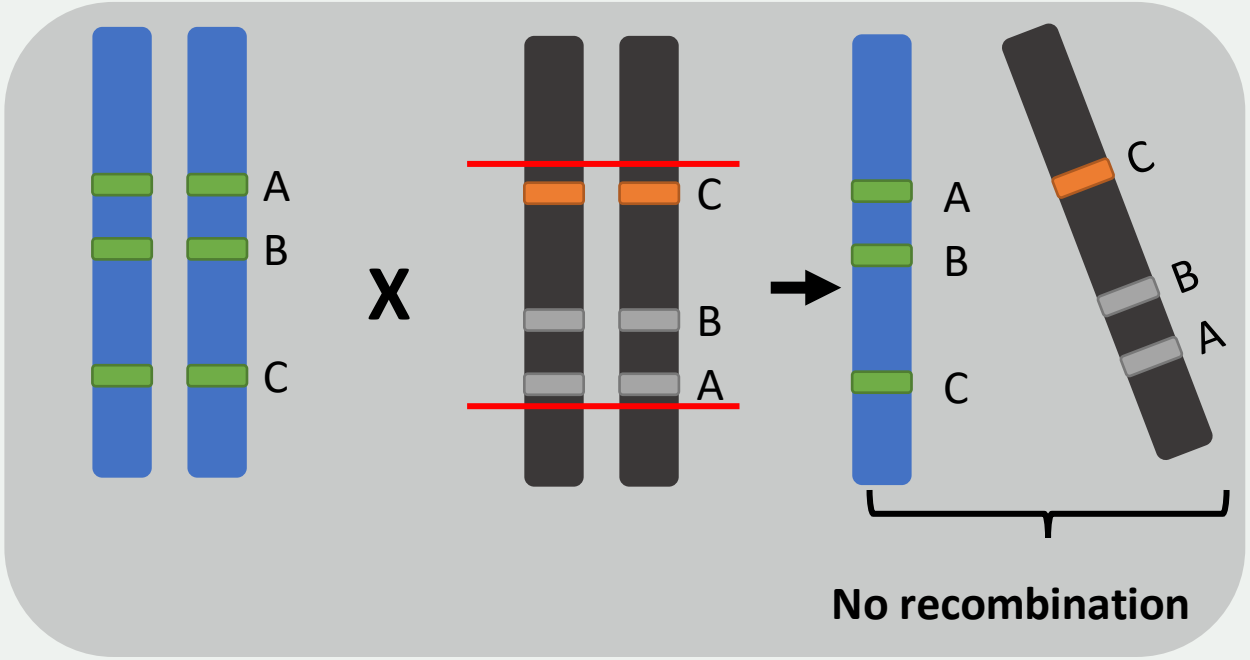
1. Cut
2. Excise
3. Inverted re-ligation

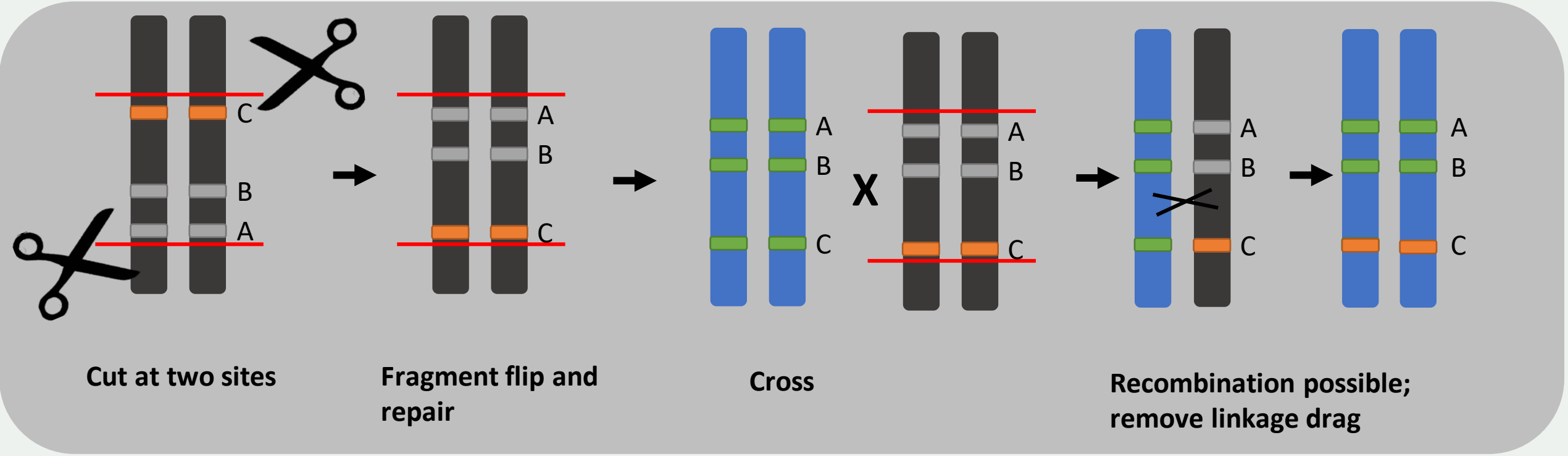


What is CRISPR-Cas?
What can we do with it?

By making an inversion, we can:

- Revert naturally occurring inversions and remove linkage drag[4]

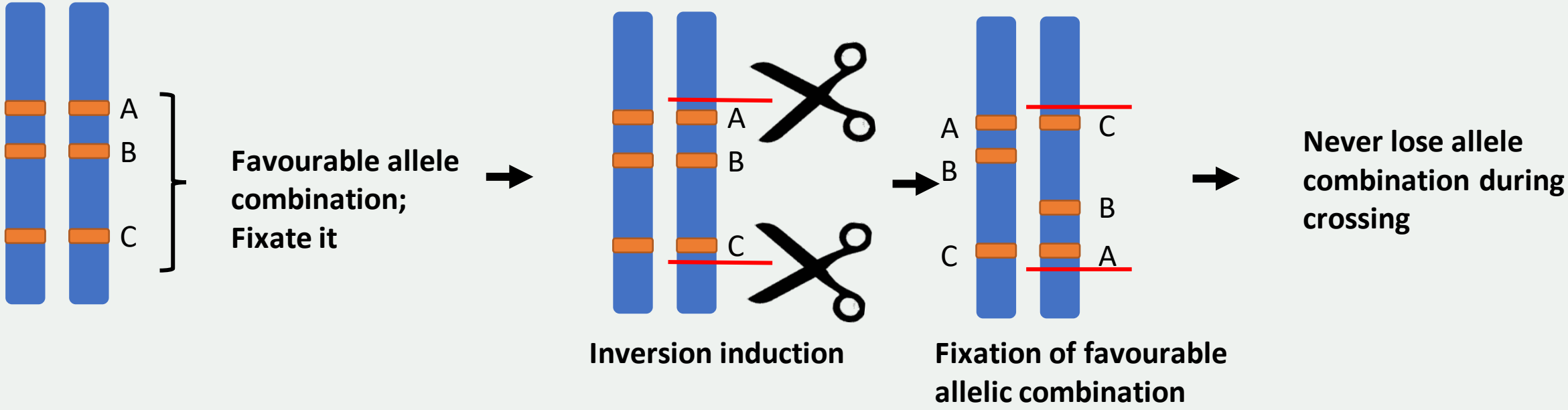




What is CRISPR-Cas? What can we do with it?

By making a an inversion, we can:

- Revert naturally occurring inversions and remove linkage drag[4]
- Fixate favourable allele combinations (suppress crossovers)[5]



4: Schmidt et al., 2020
5: Rönspies et al., 2022

What is CRISPR-Cas? What can we do with it?

By making an inversion, we can:

- Revert naturally occurring inversions and remove linkage drag
- Fixate favourable allele combinations
- Generate novel promoter-gene combinations without addition of foreign DNA[6]

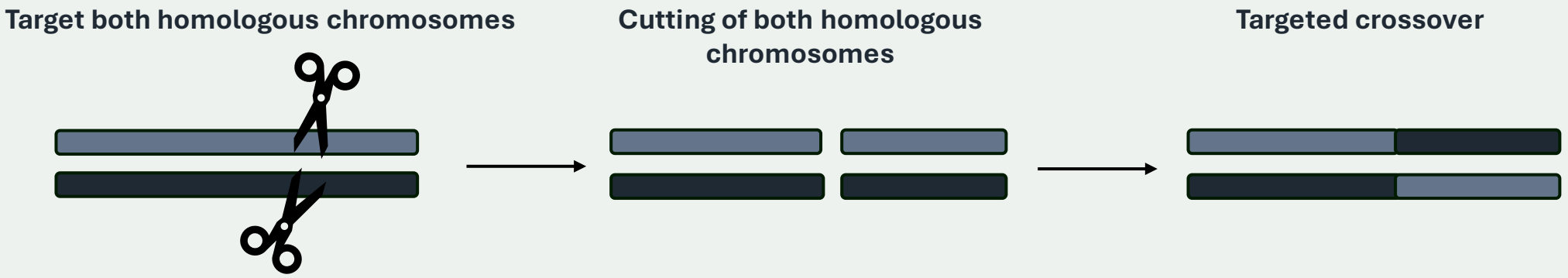


6: Lu et al., 2021

What is CRISPR-Cas? What can we do with it?

By making a precise edit, we can:

- Choose precisely where we want crossovers to occur: targeted recombination*



- Advantages: Break linkage drag, reshape allele combinations, reduce backcrossing efforts

* Filler Hayut et al., 2017 | Kouranov et al., 2022 | Grubben et al., in preparation

What is CRISPR-Cas? What can we do with it?

To summarise, by making a precise edit, we can:

1. Knock-out genes
2. Edit a promoter; alter gene expression levels
3. Induce large-scale rearrangements: Inversions, targeted recombination, etc.

Question to you: Under EU NGT legislation, will these fall under NGT1, or under NGT2?

Likely NGT1, if the inversion relates to continuous endogenous DNA and does not interrupt endogenous genes*

NGT1 covers genetic changes that could occur naturally or through conventional breeding*

* Council of the EU, *Regulation (EU) 2026/1388, OJ L, 2026/1388, 26.6.2026, Annex I.*

What is CRISPR-Cas? Beyond cutting DNA

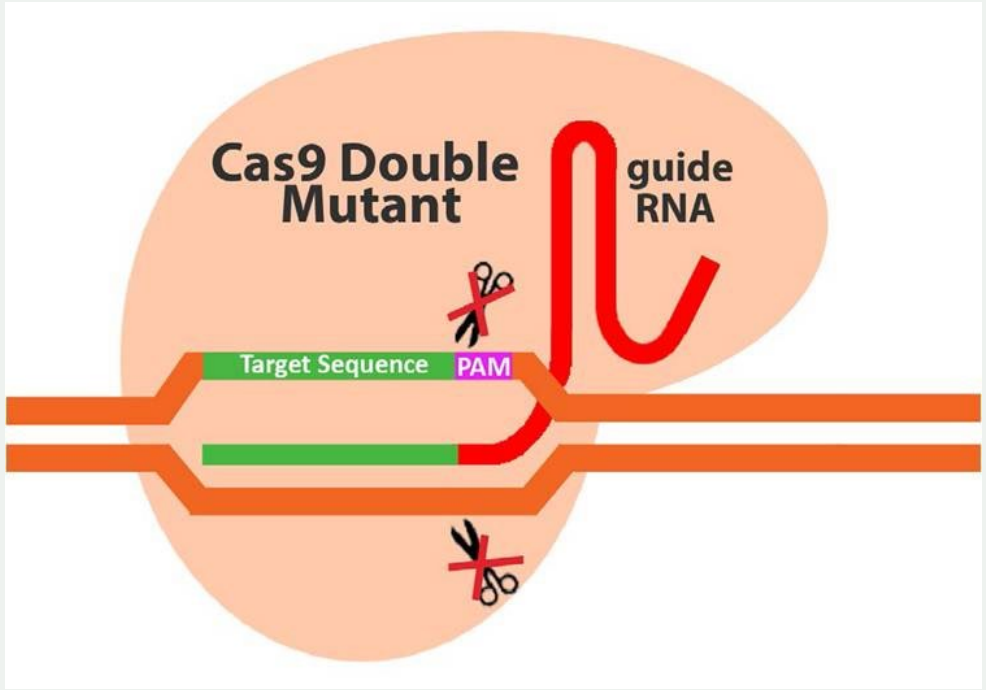
Cas nuclease protein modifications (substitutions)

- Increased fidelity; reduced off-target editing
- Increased PAM flexibility
- Increased efficiency at lower temperature (from 37°C to 25°C)
- Changing Nuclease activity: Nickases and Dead-Cas

What is CRISPR-Cas? Beyond cutting DNA

Dead- or deactivated-Cas9 (dCas9)

- Specific binding to target DNA
- No nuclease activity
- New functions: dCas9 fused to other proteins

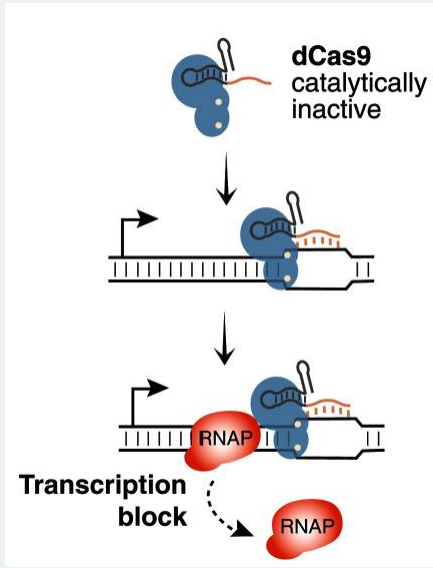


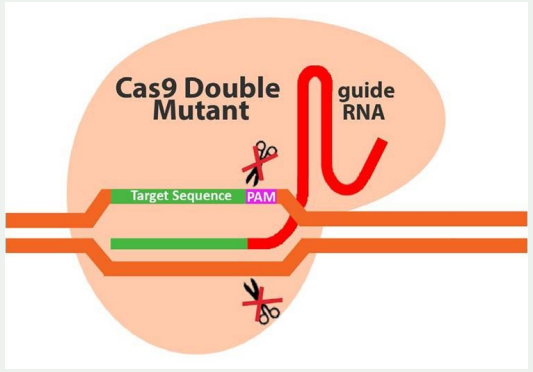
What is CRISPR-Cas? Beyond cutting DNA

Dead- or deactivated-Cas9 (dCas9)

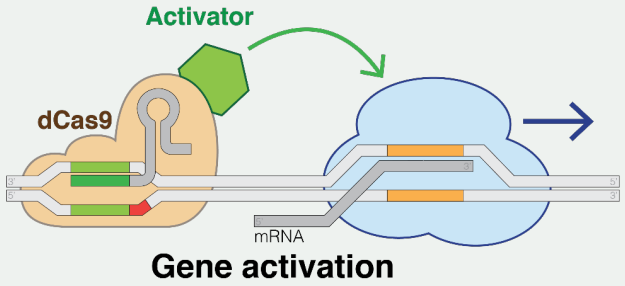
A few uses of dCas9:

1. Blocking gene expression (using just dCas9)
   Block transcription of a gene*

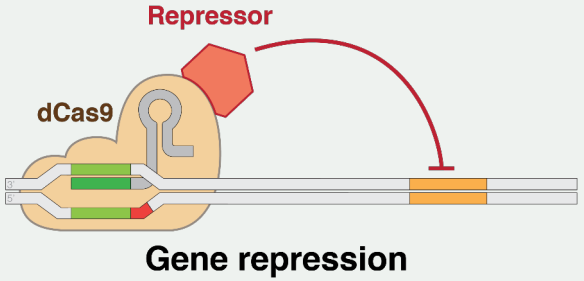




2. Targeted **activation** of gene expression**



3. Targeted **repression** of gene expression

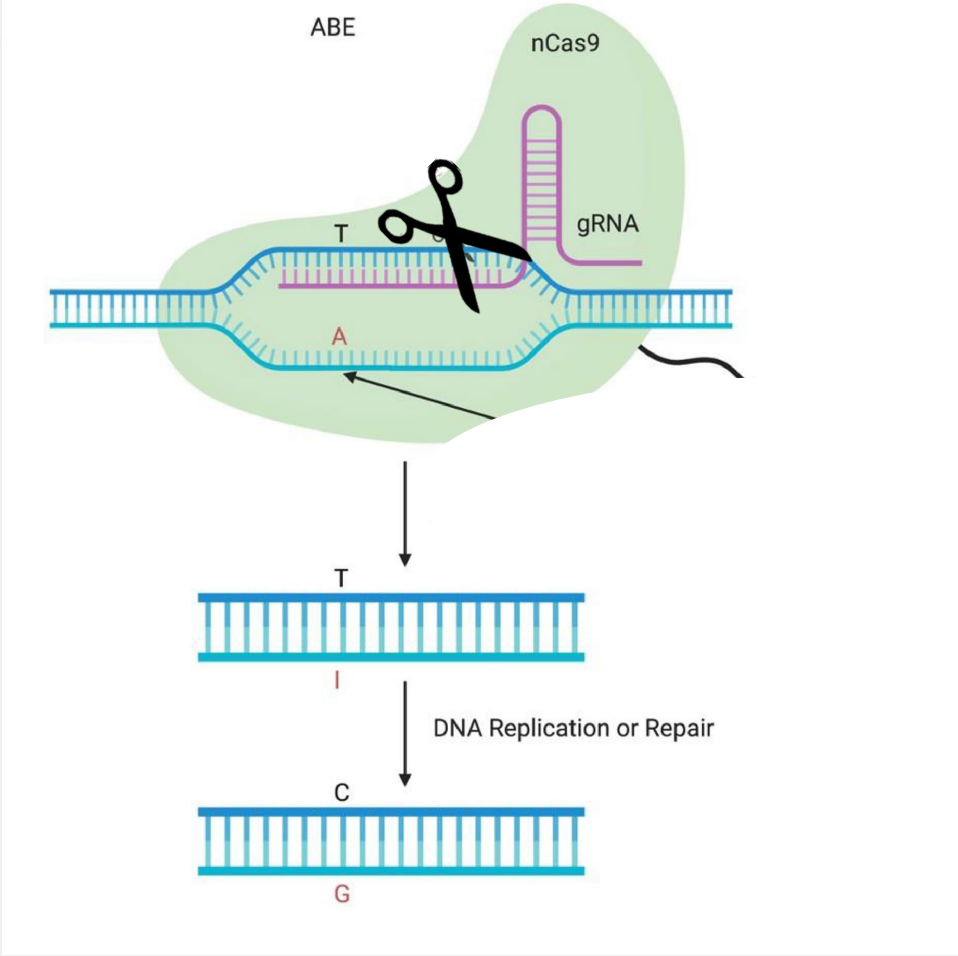


* Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). *Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression.*
**dCas9 applications (illustration CC BY-SA 4.0 Marius Walter)
https://novateinbio.com/content/96-crispr-explained

What is CRISPR-Cas? Beyond cutting DNA

Base Editors for targeted base substitutions*

- Cas9nickase (Cas9 modified to cuts single DNA strand)
- Fused to Adenosine or Cytidine Deaminase
- Converts single DNA letters (nucleotides)
- Precise mutation at predicted location

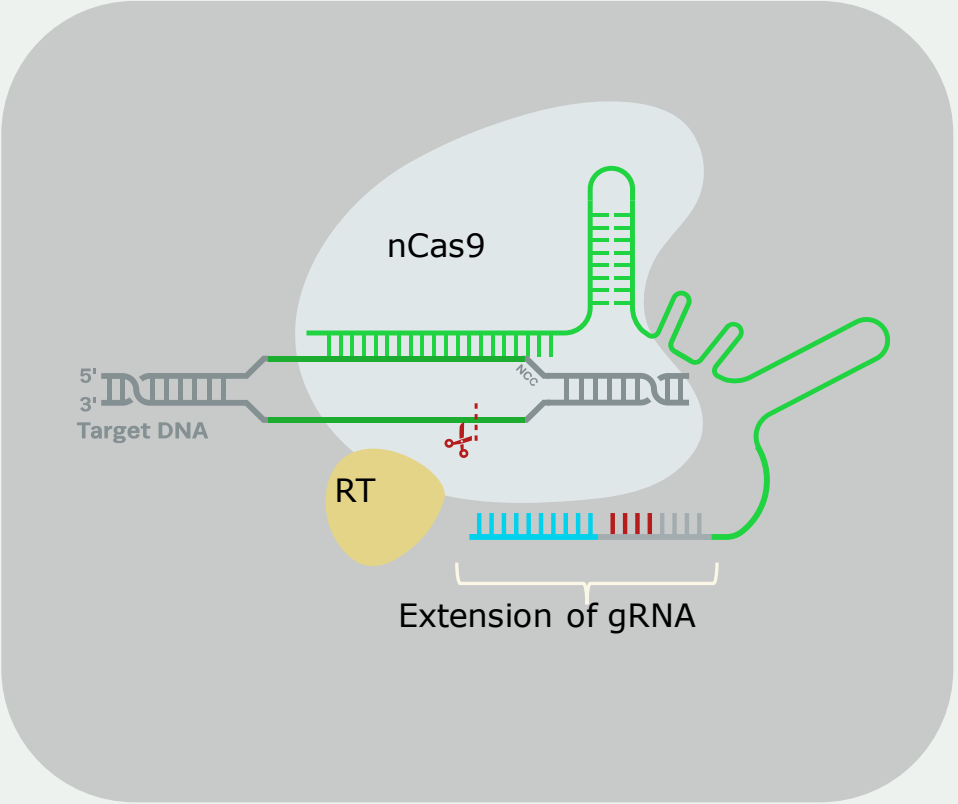


* Lahr, W. S., Sipe, C. J., Skeate, J. G., Webber, B. R., & Moriarity, B. S. (2023). *CRISPR-Cas9 base editors and their current role in human therapeutics*. Cytotherapy, 25(3), 270-276.

What is CRISPR-Cas? Beyond cutting DNA

Prime editors for rewriting short stretches of target sequence*

- Complex system
- Cas9 nickase fused to Reverse Transcriptase (RT)
- RT copies the guide RNA extension into DNA, rewriting a short stretch of the target
- Recently demonstrated to efficiently edit in dicots**



* Li, H., Chai, Z., Shi, X., Sun, C., Zhang, R., Zhang, Q., ... & Gao, C. (2026). *Multiplexed, precise genome engineering in monocots with twin prime editing systems.* Nature Biotechnology, 1-10.
** Liu, Y., Sun, C., Xu, H., Ran, Y., Li, L., Zhang, R., & Gao, C. (2026). *SmartPE enables efficient prime editing in dicot crops.* Nature Plants, 1-8.

ThermoCas9 development for plant breeding

A thermostable Cas9 with temperature-dependent activity

2015

Discovery by the van der Oost team

Isolated from a compost heap near Wageningen

2023

Initial plant studies reveal the constraint

Limited activity at standard plant temperatures

Protein engineering

Engineered variants showed improved activity at moderate temperatures

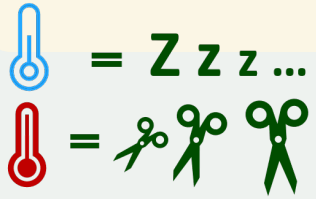
2025

Competitive editing demonstrated

Optimised ThermoCas variants performs comparably to SpCas9 in tomato

Temperature-inducible editing

Control over editing activity by altering temperature



2026 and beyond

Validation in multiple crops

Efficient editing in monocots and dicots

CRISPR4All initiative

Make CRISPR-ThermoCas technology available to all breeders, at a fair price

Supported by

biotech booster

novo nordisk foundation

Benefitting people and society

GATES AG ONE

Innovative Genomics Institute

CRISPR-Cas: An outlook

New Genomic Techniques (NGTs) accelerate breeding speed and precision

- CRISPR-Cas works best alongside established breeding methods and practices: a powerful complement, not a silver bullet
- The technology is ready
- **What about adoption of the tech?**

Rapport Wennink[1] The Netherlands has a world-class plant-breeding ecosystem, but must invest and remove regulatory barriers to stay ahead

Otherwise: Innovation and production will move to other countries

- Legislation barrier: positive outlook
- Adoption barriers?

1 https://www.rapportwennink.nl/; Commissioned by the Dutch government

CRISPR-Cas: An outlook

Adoption barriers

My view: for most breeding companies, the regulation matters less than the patent landscape

- SpCas9 is well established, but licence fees price out SMEs and the use clauses are restrictive

- SpCas9 alternatives: potentially cheaper licences with better terms, but uncertain freedom to operate

  And the breeder, not the licensor, carries the liability

  What would accelerate broad adoption?

- An affordable gene editing platform with simple terms and strong foundational patents

To Summarise

- CRISPR-Cas originates from a bacterial defence system against viruses
- Can be programmed to target specific DNA sequences
- Enables targeted genetic changes for plant breeding
- Precise breeding tool
- WURs own CRISPR enzyme: ThermoCas9
- The technology is there, broad adoption requires IP clarity and straightforward licensing terms

Thank you for your attention!

Questions?