LGN0232 - Genética Molecular

Técnicas Inovadoras de Melhoramento de Precisão –TIMPs

CRISPR & RNAi

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Técnicas Inovadoras de Melhoramento de Precisão - TIMPs

- Definições de Organismos Geneticamente Modificados e Transgenia

- Protocolo de Cartagena de Biossegurança define LMO como "*any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology*." — ***L**iving **M**odified **O**rganism*

- Novas metodologias ou abordagens que resultam na **AUSÊNCIA de DNA/RNA recombinante** no produto final

Todo Organismo Geneticamente Modificado (OGM) é um Transgênico?

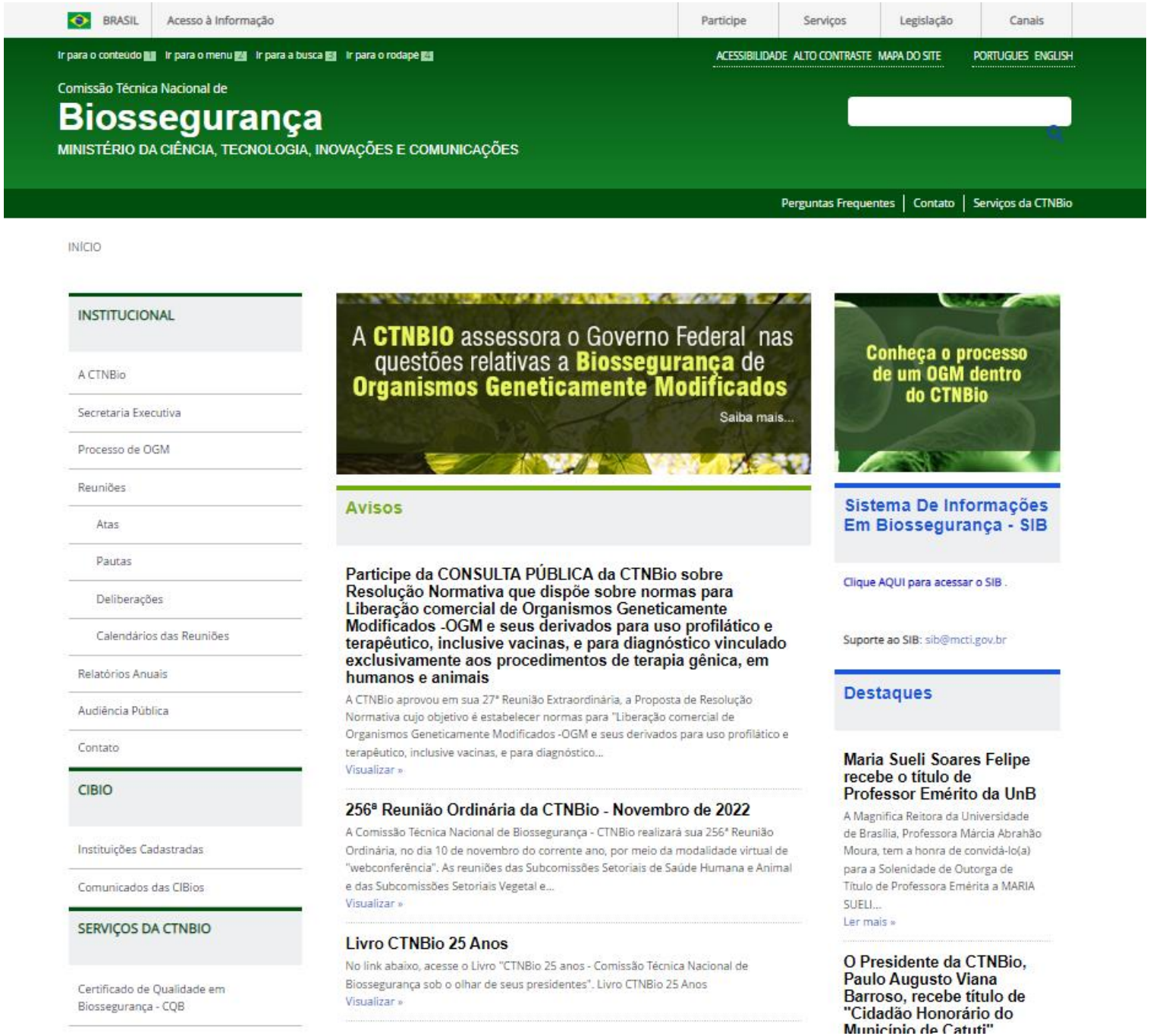
Quem controla a liberação de Organismos Geneticamente Modificados?

Foto: Paulo Barroso

Eles são seguros?

CTNBIO

http://ctnbio.mctic.gov.br/inicio



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Plantas Plantas Subpastas: Algodão, Cana, Eucalipto, Farinha de Trigo, Feijão, Mais »
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English Version Subpastas: Crops, Microorganisms, Others, Vacccines
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Mostrando 6 resultados. Itens por página 50 **Página** Primeiro | Anterior | Próximo | Último

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Plantas

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Tabela de Plantas - Uso Comercial Plantas Geneticamente modificadas aprovadas para Comercialização
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Farinha de Trigo Subpastas: Parecer Técnico nº 7795 - 2021
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Cana Subpastas: Parecer Técnico nº 5483 - 2017, Parecer Técnico nº 6235 - 2018, Parecer Técnico nº 6658 - 2019, Parecer Técnico nº 7140 - 2020, Parecer Técnico nº 7246 - 2020, Mais »
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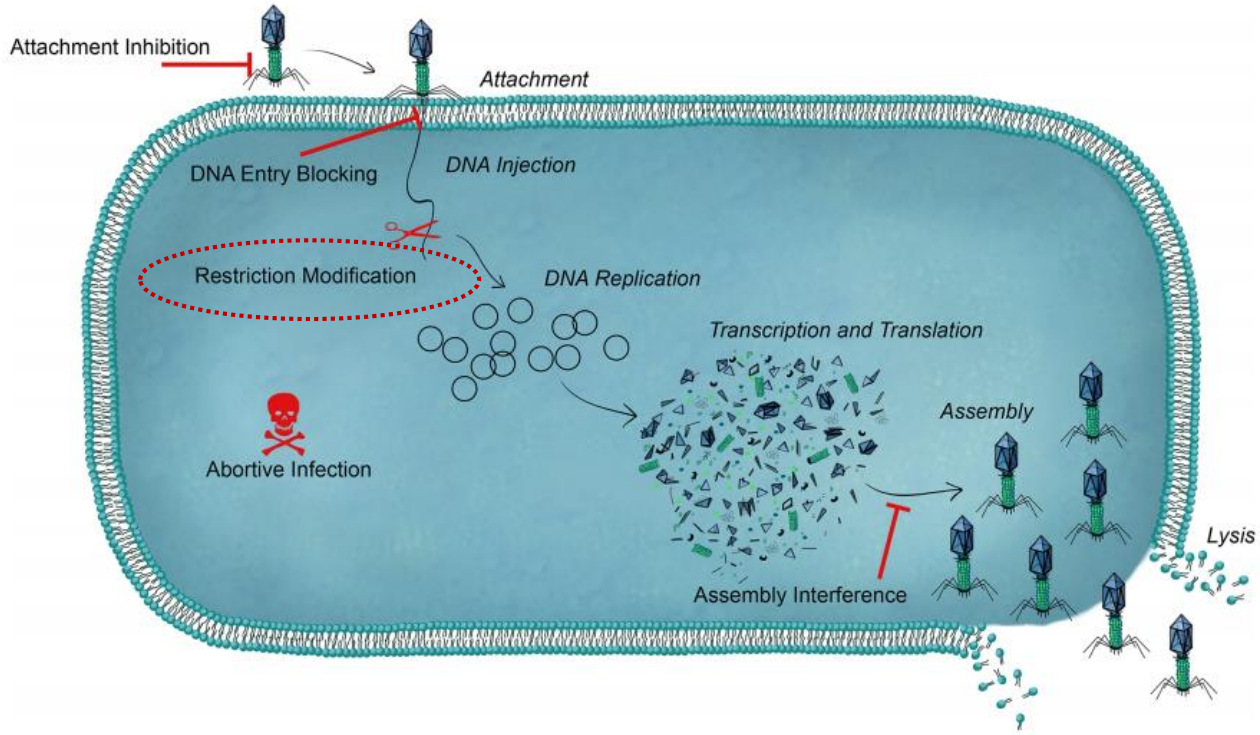
Mostrando 8 resultados. Itens por página 50 **Página** 1 de 1 Primeiro | Anterior | Próximo | Último

Técnicas Inovadoras de Melhoramento de Precisão

CRISPR/Cas – Edição Genômica

Alguns mecanismos de defesa de bactérias inatos …



PLOS | PATHOGENS

PEARLS

Battling Phages: How Bacteria Defend against Viral Attack

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Introduction

Bacteriophages (phages) are accomplished, bacteria-specific, viral predators with far-reaching impact: from the food and biotechnology industries [1] to global nutrient cycling [2] to human health and disease [3]; wherever bacteria thrive, it seems, so do predatory phages. In order to survive the constant onslaught of phage, bacteria have evolved mechanistically diverse defense strategies that act at every stage of the phage life cycle (Fig 1) [4,5]. Phages rapidly co-evolve to overcome these barriers, resulting in a constant, and often surprising, molecular arms race [6]. In this review, I highlight the spectrum of "innate" strategies used by bacteria to evade phage predation, with particular attention paid to more recent findings in the field. For a discussion of the CRISPR-Cas adaptive immune system, readers are directed to several recent reviews [4–6].

E o CRISPR-Cas?

JOURNAL OF BACTERIOLOGY, June 1989, p. 3553–3556
0021-9193/89/063553-04$02.00/0

Vol. 171, No. 6

Unusual Nucleotide Arrangement with Repeated Sequences in the *Escherichia coli* K-12 Chromosome

ATSUO NAKATA,* MITSUKO AMEMURA, AND KOZO MAKINO

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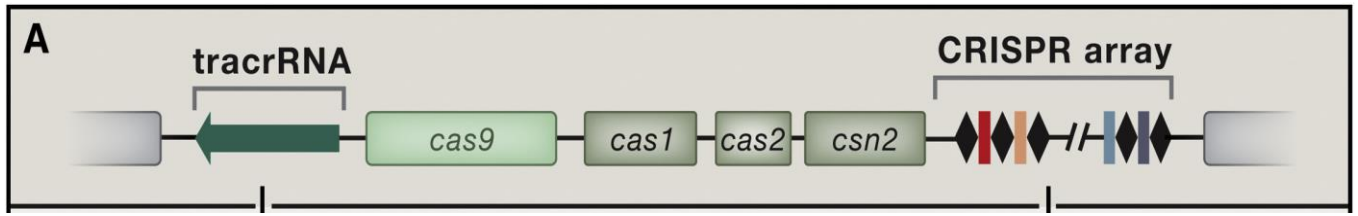
Received 19 December 1988/Accepted 13 March 1989

Between 59 and 60 min on the *Escherichia coli* genetic map, there is a highly conserved sequence of 29 base pairs, containing an inverted repeat of seven base pairs that appears 14 times, 32 or 33 base pairs apart, downstream of the *iap* gene coding region. About 24 kilobase pairs downstream of the 14 repeats, a similar 29-base-pair sequence with a spacing of 32 base pairs appears seven times. Nucleotide sequences hybridizing with the 29-base-pair fragment were also detected in *Shigella dysenteriae* and *Salmonella typhimurium* but not in *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*.

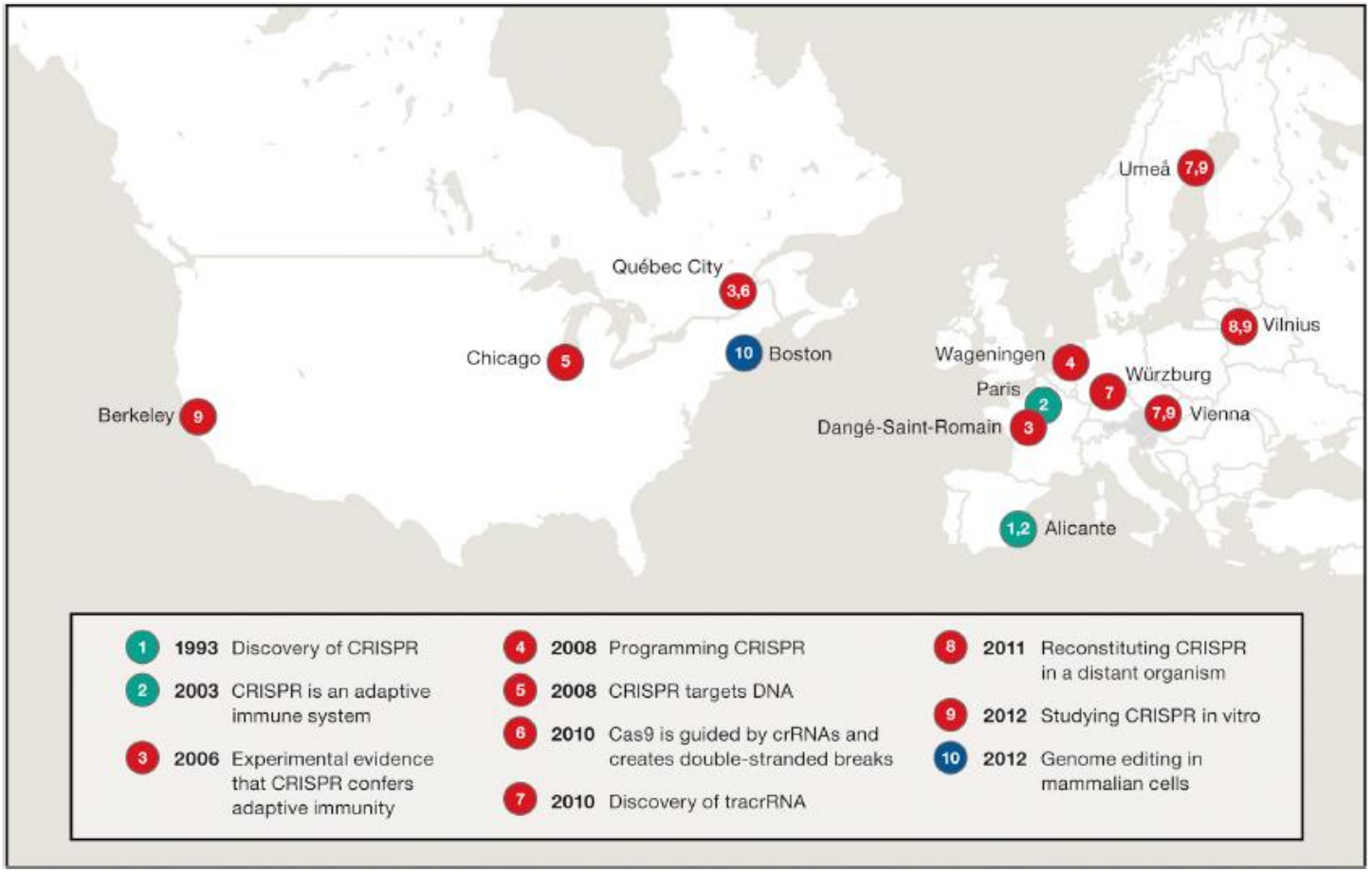
…14 repeats of 29 base pairs (bp) that were interspersed by 32–33 bp non-repeating spacer sequences….

Definição de CRISPR

CRISPR–Cas (*Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-associated proteins Cas*) -> módulos = sistema adaptativos de imunidade presente em muitas arqueias e bactérias



CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) + Cas (**CR**ISPR-**as**sociated)



https://www.cell.com/fulltext/S0092-8674%2815%2901705-5

The history also illustrates the growing role in biology of "hypothesis-free" discovery based on big data. The discovery of the CRISPR loci, their biological function, and the tracrRNA all emerged not from wet-bench experiments but from open-ended bioinformatic exploration of large-scale, often public, genomic datasets. "Hypothesis-driven" science of course remains essential, but the 21st century will see an increasing partnership between these two approaches.

It is instructive that so many of the Heroes of CRISPR did their seminal work near the very start of their scientific careers (including Mojica, Horvath, Marraffini, Charpentier, Vogel, and Zhang)—in several cases, before the age of 30. With youth often comes a willingness to take risks—on uncharted directions and seemingly obscure questions—and a drive to succeed. It's an important reminder at a time that the median age for first grants from the NIH has crept up to 42.

Notably, too, many did their landmark work in places that some might regard as off the beaten path of science (Alicante, Spain; France's Ministry of Defense; Danisco's corporate labs; and Vilnius, Lithuania). And, their seminal papers were often rejected by leading journals—appearing only after considerable delay and in less prominent venues. These observations may not be a coincidence: the settings may have afforded greater freedom to pursue less trendy topics but less support about how to overcome skepticism by journals and reviewers.

Finally, the narrative underscores that scientific breakthroughs are rarely eureka moments. They are typically ensemble acts, played out over a decade or more, in which the cast becomes part of something greater than what any one of them could do alone. It's a wonderful lesson for the general public, as well as for a young person contemplating a life in science.

Trabalho pioneiro sobre CRISPR/Cas....

Molecular Microbiology (1993) **9**(3), 613–621

Transcription at different salinities of *Haloferax mediterranei* sequences adjacent to partially modified *Pst*I sites

F. J. M. Mojica, G. Juez and F. Rodríguez-Valera*
Departamento de Genética Molecular y Microbiología, Apartado 374, Universidad de Alicante, 03080 Alicante, Spain.

Summary

Two genomic sequences from the halophilic archaeon *Haloferax mediterranei*, where we had found *Pst*I restriction-pattern modifications depending on the salinity of the growth medium, have been studied. A markedly salt-dependent differential expression has been detected in the nearby regions. Two of the open reading frames characterized correspond to two of the differentially expressed transcripts. In both cases the *Pst*I sites were included in purine–pyrimidine alternancies suggestive of Z-DNA structures and located in non-coding regions with frequent repetitive motifs. A long alternating adenine–thymine tract also appears in the upstream regions of one of these open reading frames. A possible role of local DNA configuration in osmoregulation in this organism is discussed.

involved in the high-affinity K^+ transport, whose regulation is effected at transcriptional level and is being extensively studied (Csonka, 1989; May *et al.*, 1989; Mizuno and Mizushima, 1990; Sugiura *et al.*, 1992). A role for the topology of DNA and intracellular K^+ concentrations in osmoregulation has been suggested (Sutherland *et al.*, 1986; Higgins *et al.*, 1987; 1988; Graeme-Cook *et al.*, 1989; Ramirez and Villarejo, 1991). In the case of halobacteria there is little evidence of the effect of salinity on gene expression. To our knowledge, the only reference to the subject concerns a markedly different expression of the *mc-vac* gene encoding the major gas vesicles protein of *Haloferax mediterranei* at different salinities (Englert *et al.*, 1990).

We previously described the existence of certain *Pst*I sites in the *H. mediterranei* genome which appeared to be more susceptible to cleavage, or less, depending on the salt concentration at which the cells were grown (Juez *et al.*, 1990). At least 5% of the clones from a genomic library of the organism used as probes revealed restriction-pattern modifications which appeared to be consistently associated with the salinity of the growth medium. To clarify whether this phenomenon could have any biological significance implicated in the adaptation of the

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

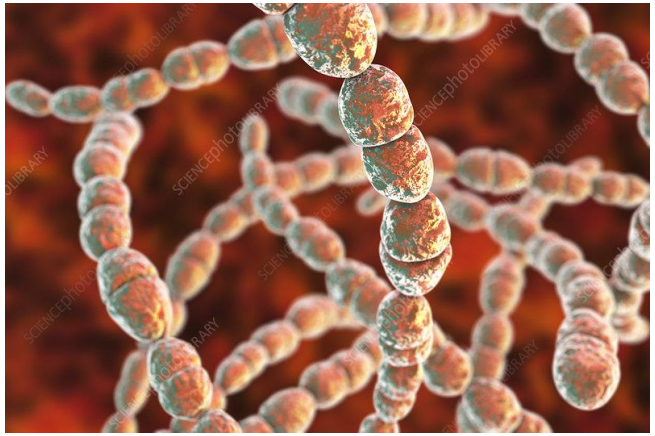
Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*

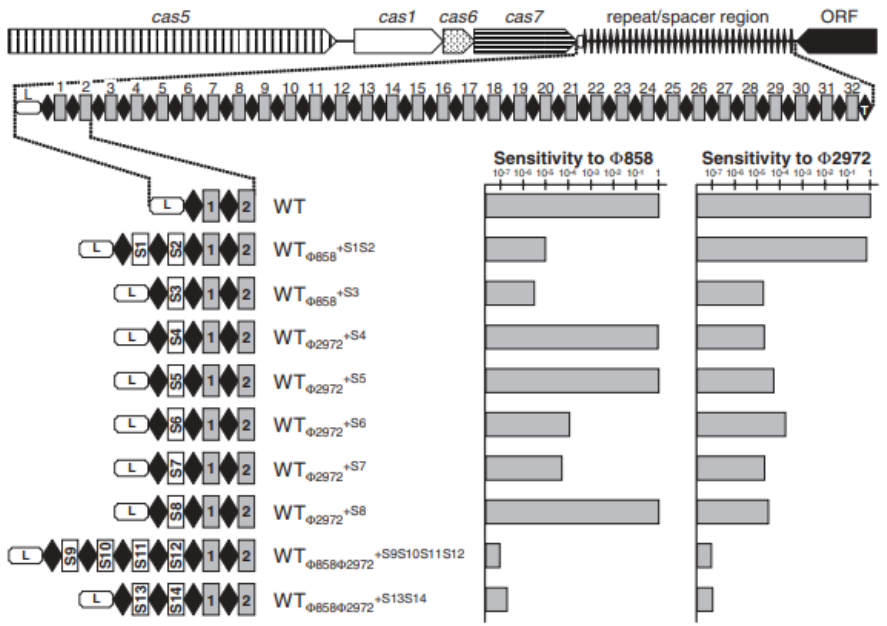
Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences. 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.

Definiram função de CRISPR

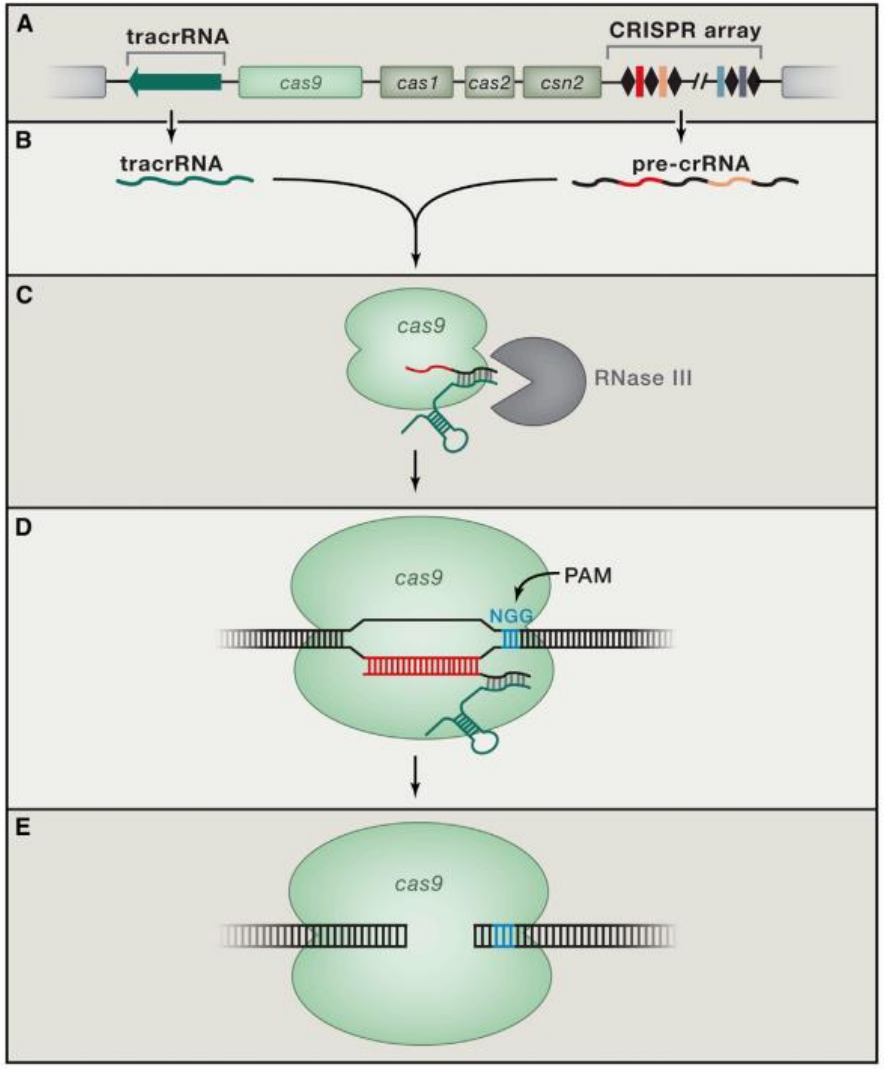
Science 2007 315,1709-1712

Ensaio com *Streptococcus thermophilus*





CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) + Cas (**CRISPR-as**sociated)



Sistema Imune Adaptativo

Figure 1. Class 2, Type II CRISPR-Cas9 System from *Streptococcus thermophilus*

Type II systems are the simplest of the three types of CRISPR systems and have been the basis for genome editing technology.

(A) The locus contains a CRISPR array, four protein-coding genes (*cas9*, *cas1*, *cas2*, and *cns2*) and the tracrRNA. The CRISPR array contains repeat regions (black diamonds) separated by spacer regions (colored rectangles) derived from phage and other invading genetic elements. The *cas9* gene encodes a nuclease that confers immunity by cutting invading DNA that matches existing spacers, while the *cas1*, *cas2*, and *cns2* genes encode proteins that function in the acquisition of new spacers from invading DNA.

(B) The CRISPR array and the tracrRNA are transcribed, giving rise to a long pre-crRNA and a tracrRNA.

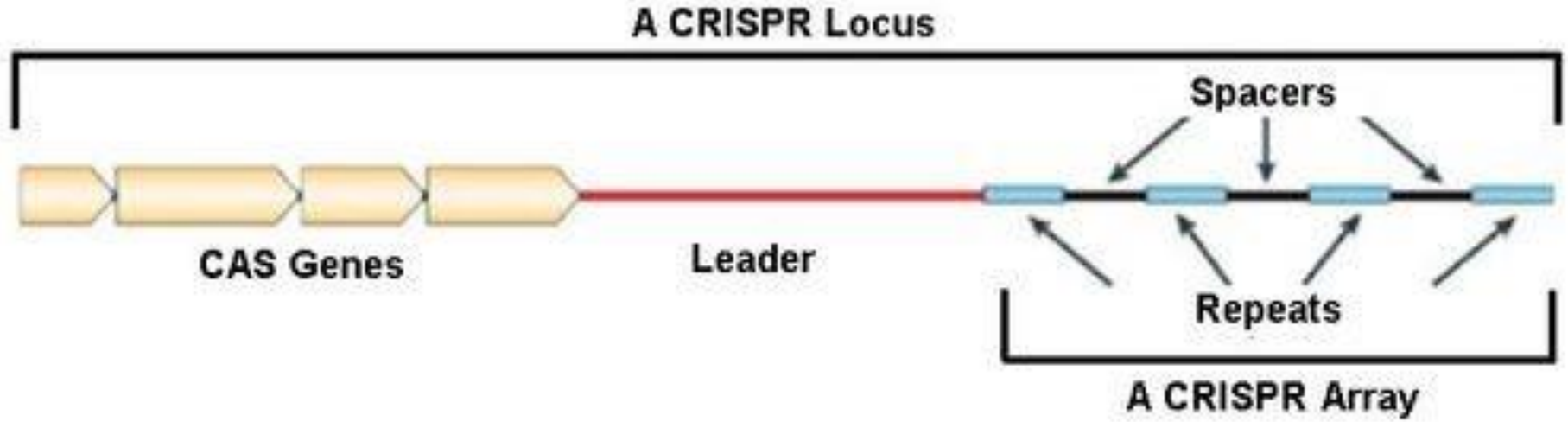
(C) These two RNAs hybridize via complementary sequences and are processed to shorter forms by Cas9 and RNase III.

(D) The resulting complex (Cas9 + tracrRNA + crRNA) then begins searching for the DNA sequences that match the spacer sequence (shown in red). Binding to the target site also requires the presence of the protospacer adjacent motif (PAM), which functions as a molecular handle for Cas9 to grab on to.

(E) Once Cas9 binds to a target site with a match between the crRNA and the target DNA, it cleaves the DNA three bases upstream of the PAM site. Cas9 contains two endonuclease domains, HNH and RuvC, which cleave, respectively, the complementary and non-complementary strands of the target DNA, creating blunt ends.

Definição de CRISPR

CRISPR–Cas (***C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats – CRISPR-associated proteins Cas*) = módulos são sistema adaptativos de imunidade presente em muitas arquéias e bactérias



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]‡

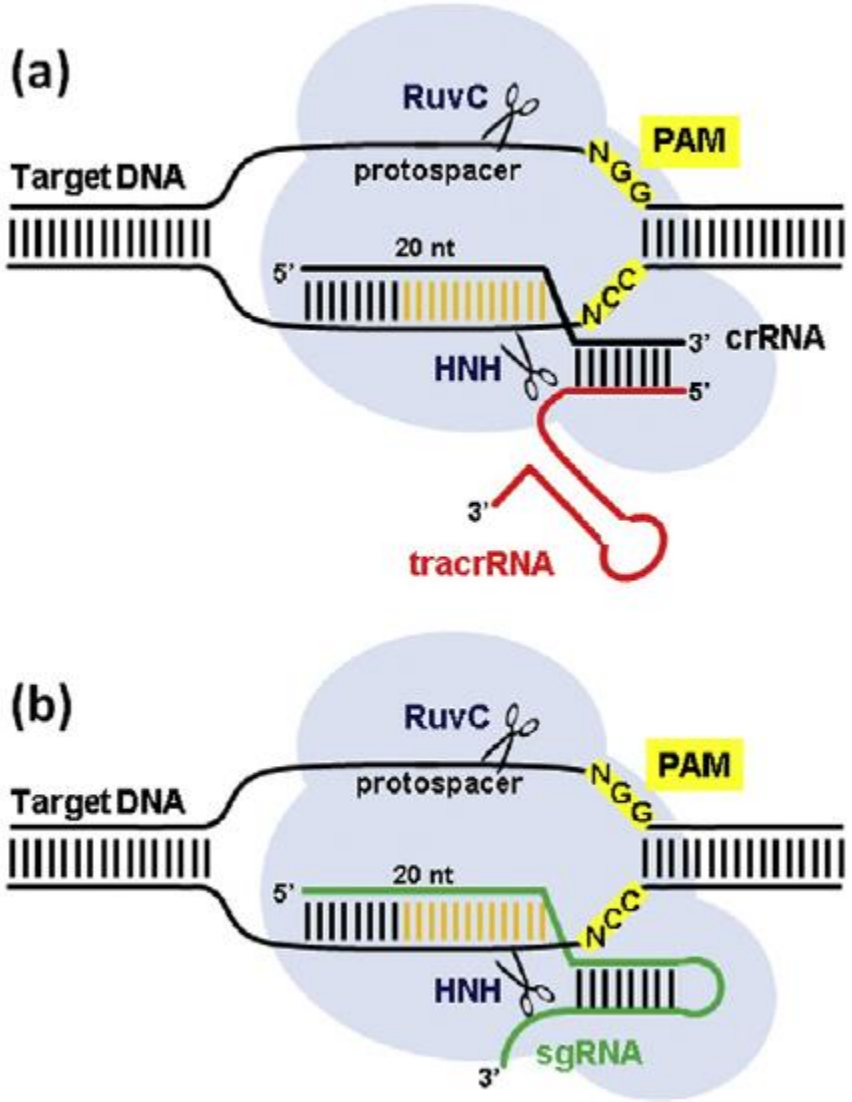
Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, 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.

Streptococcus pyogenes

Science 337, 816-821 DOI: 10.1126/science.1225829

Jennifer Doudna e Emmanuelle Charpentier, vencedoras do Prêmio Nobel de Química 2020 (Foto: Alexander Heinl/picture alliance via Getty Images)

PRÊMIO NOBEL DE QUIMICA EM 2020



doi: 10.1016/j.biotechadv.2014.12.006

Otimização do Sistema Crispr-Cas9

CRISPR RNA (crRNA)

***trans*-acting CRISPR RNA (tracrRNA)**

crRNA + tracRNA -> sgRNA

Protospacer Adjacent Motif (PAM)

CRISPR em Mamíferos e Humanos em 2013

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*).

The *Streptococcus pyogenes* SF370 type II CRISPR locus consists of four genes, including the Cas9 nuclease, as well as two noncoding CRISPR RNAs (crRNAs): trans-activating crRNA (tracrRNA) and a precursor crRNA (pre-crRNA) array containing nuclease guide sequences (spacers) interspaced by identical direct repeats (DRs) (fig. S1) (*19*). We sought to harness this prokaryotic

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†To whom correspondence should be addressed. E-mail: zhang@broadinstitute.org

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 reconstitution of the *Streptococcus pyogenes* type II CRISPR system demonstrated that crRNA fused to a normally trans-encoded tracrRNA is sufficient to direct Cas9 protein to sequence-specifically cleave target DNA sequences matching the crRNA (*4*). The fully defined nature of this two-component system suggested that it might function in the cells of eukaryotic organisms such as yeast, plants, and even mammals. By cleaving genomic sequences targeted by RNA sequences (*4*–*6*), such a system could greatly enhance the ease of genome engineering.

Here, we engineer the protein and RNA components of this bacterial type II CRISPR system in human cells. We began by synthesizing a human codon–optimized version of the Cas9 protein bearing a C-terminal SV40 nuclear localization signal and cloning it into a mammalian expression system (Fig. 1A and fig. S1A). To direct Cas9 to cleave sequences of interest, we expressed crRNA-tracrRNA fusion transcripts, hereafter referred to as guide RNAs (gRNAs), from the human U6 polymerase III promoter. Directly transcribing gRNAs allowed us to avoid reconstituting the RNA-processing machinery used by bacterial CRISPR systems (Fig. 1A and fig. S1B) (*4*, *7*–*9*). Constrained only by U6 transcription initiating with G and the requirement for the PAM (protospacer-adjacent motif) sequence -NGG following the 20–base pair (bp) crRNA target, our highly versatile approach can, in principle, target any genomic site of the form $GN_{20}GG$ (fig. S1C; see supplementary text S1 for a detailed discussion).

To test the functionality of our implementation for genome engineering, we developed a green fluorescent protein (GFP) reporter assay (Fig. 1B) in human embryonic kidney HEK 293T cells similar to one previously described (*10*). Specifically, we established a stable cell line bearing a genomically integrated GFP coding sequence disrupted by the insertion of a stop codon and a 68-bp genomic fragment from the AAVS1 locus that renders the expressed protein fragment nonfluorescent. Homologous recombination (HR) using an appropriate repair donor can restore the normal GFP sequence, which enabled us to quantify the resulting GFP^+ cells by flow-activated cell sorting (FACS).

To test the efficiency of our system at stimulating HR, we constructed two gRNAs, T1 and T2, that target the intervening AAVS1 fragment (Fig. 1B) and compared their activity to that of a previously described TAL effector nuclease heterodimer (TALEN) targeting the same region (*11*). We observed successful HR events using all three targeting reagents, with gene correction rates using the T1 and T2 gRNAs approaching 3% and 8%, respectively (Fig. 1C). This RNA-mediated editing process was notably rapid, with the first detectable GFP^+ cells appearing ~20 hours post transfection compared with ~40 hours for the AAVS1 TALENs. We observed HR only upon simultaneous introduction of the repair donor, Cas9 protein, and gRNA, which confirmed that all components are required for genome editing (fig. S2). Although we noted no apparent toxicity associated with Cas9/gRNA expression, work with zinc finger nucleases (ZFNs) and TALENs has shown that nicking only one strand further reduces toxicity. Accordingly, we also tested a Cas9D10A mutant that is known to function as a nickase in vitro, which yielded similar HR but lower nonhomologous end joining (NHEJ) rates (fig. S3) (*4*, *5*). Consistent with (*4*), in which a related Cas9 protein is shown to cut both strands

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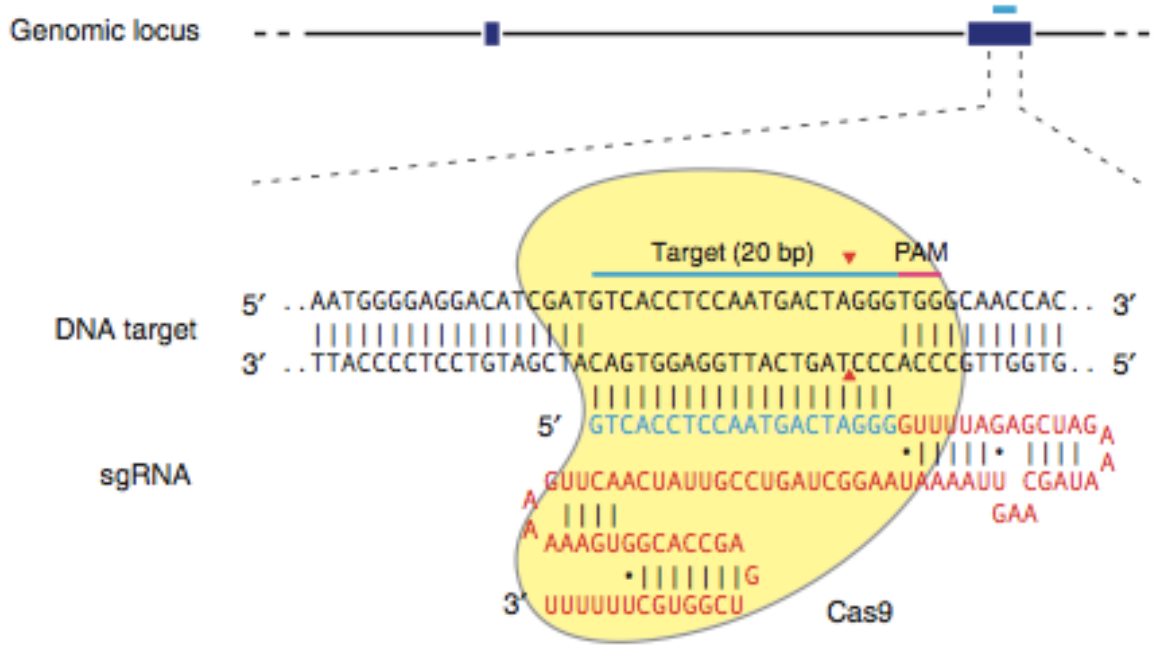
CRISPR-Cas9

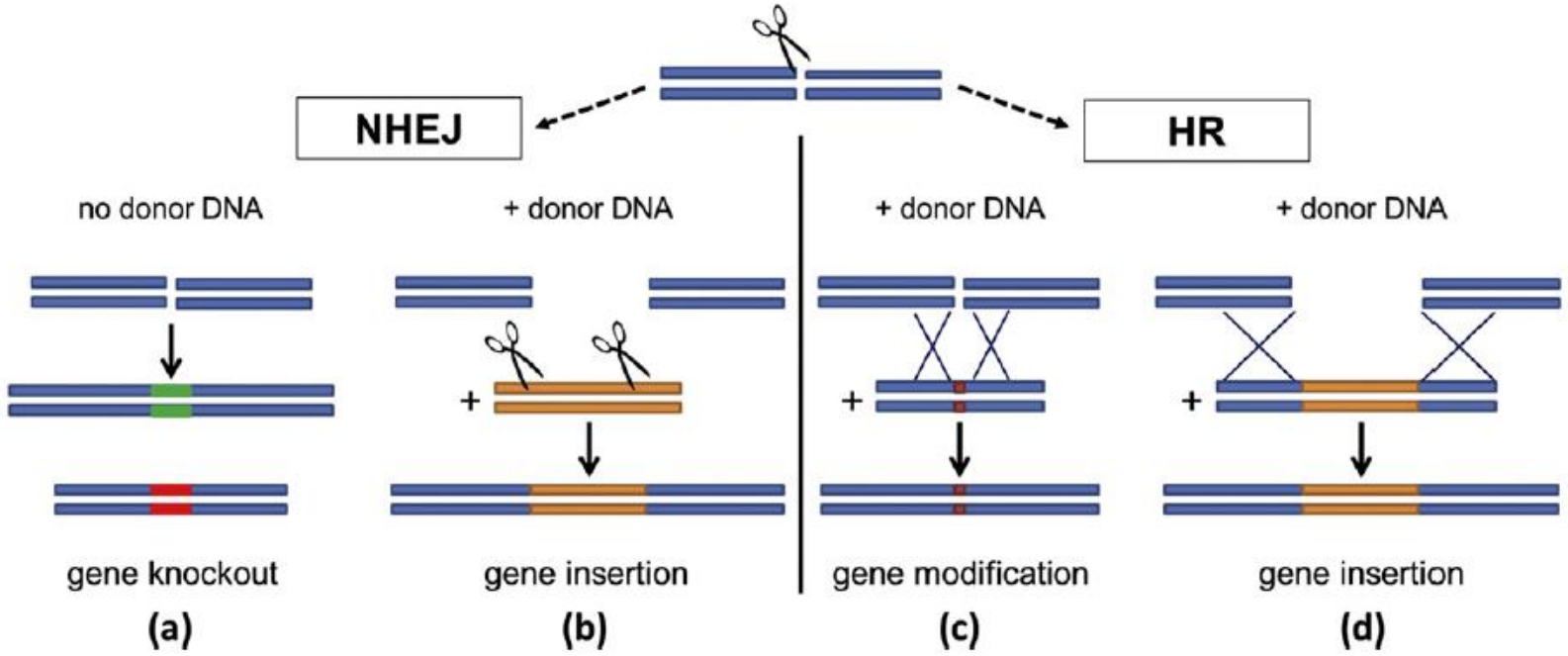
CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) + Cas (**CR**ISPR-**as**sociated)



https://www.youtube.com/watch?v=MnYppmstxIs

https://www.youtube.com/watch?v=2pp17E4E-O8

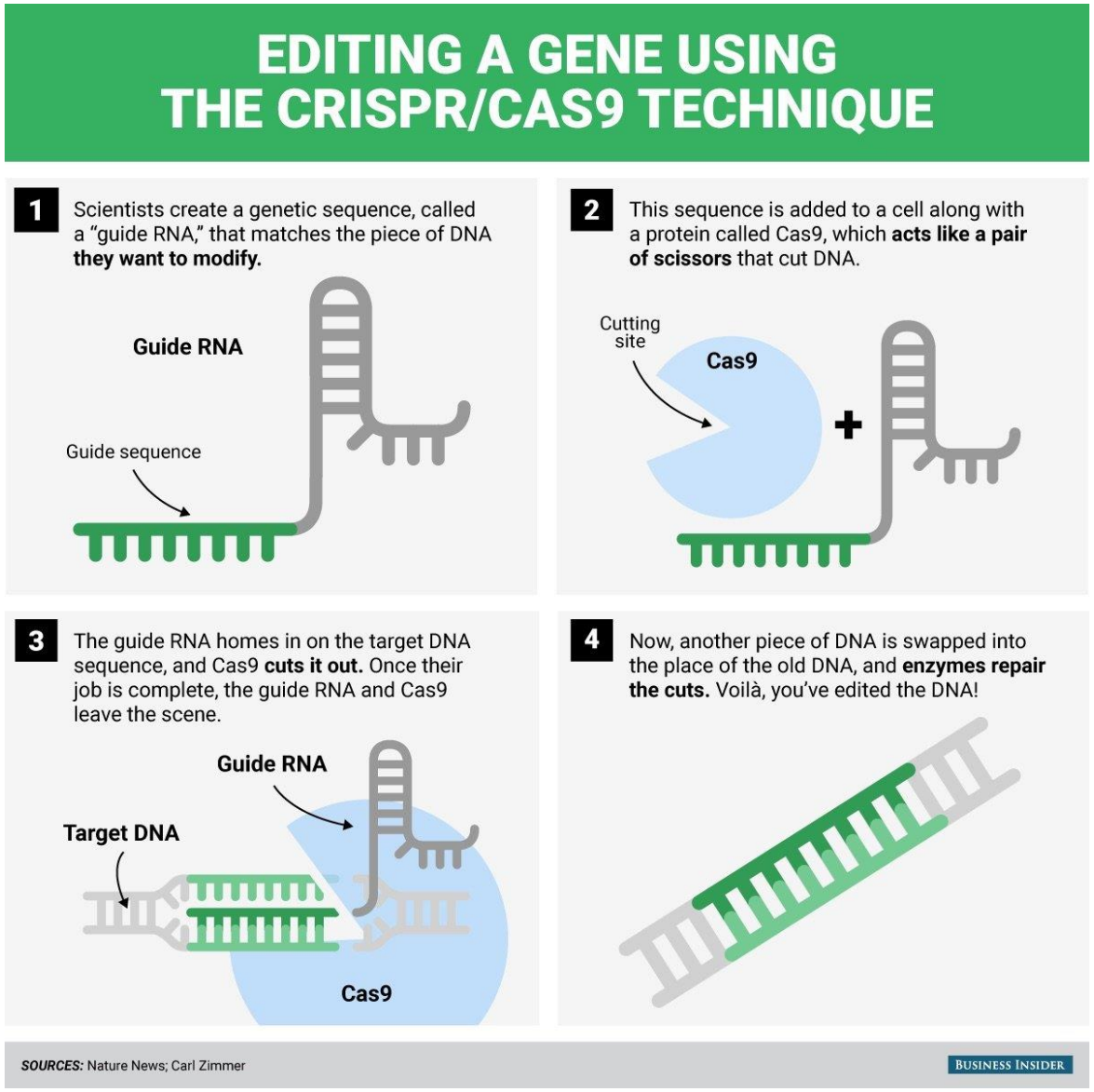
SISTEMAs DE RECOMBINAÇÃO – em CRISPR/Cas



NHEJ = *Non-homologous end joining* (a introdução de inserções ou deleções na sequencia alvo)

HR = *homologous recombination* (troca de informação genética entre moléculas de DNA com sequências similares)

Doi: 10.1016/j.biotechadv.2014.12.006



https://www.youtube.com/watch?v=47pkFey3CZ0

https://www.youtube.com/watch?v=TdBAHexVYzc

Edição Genômica – CRISPR/Cas9

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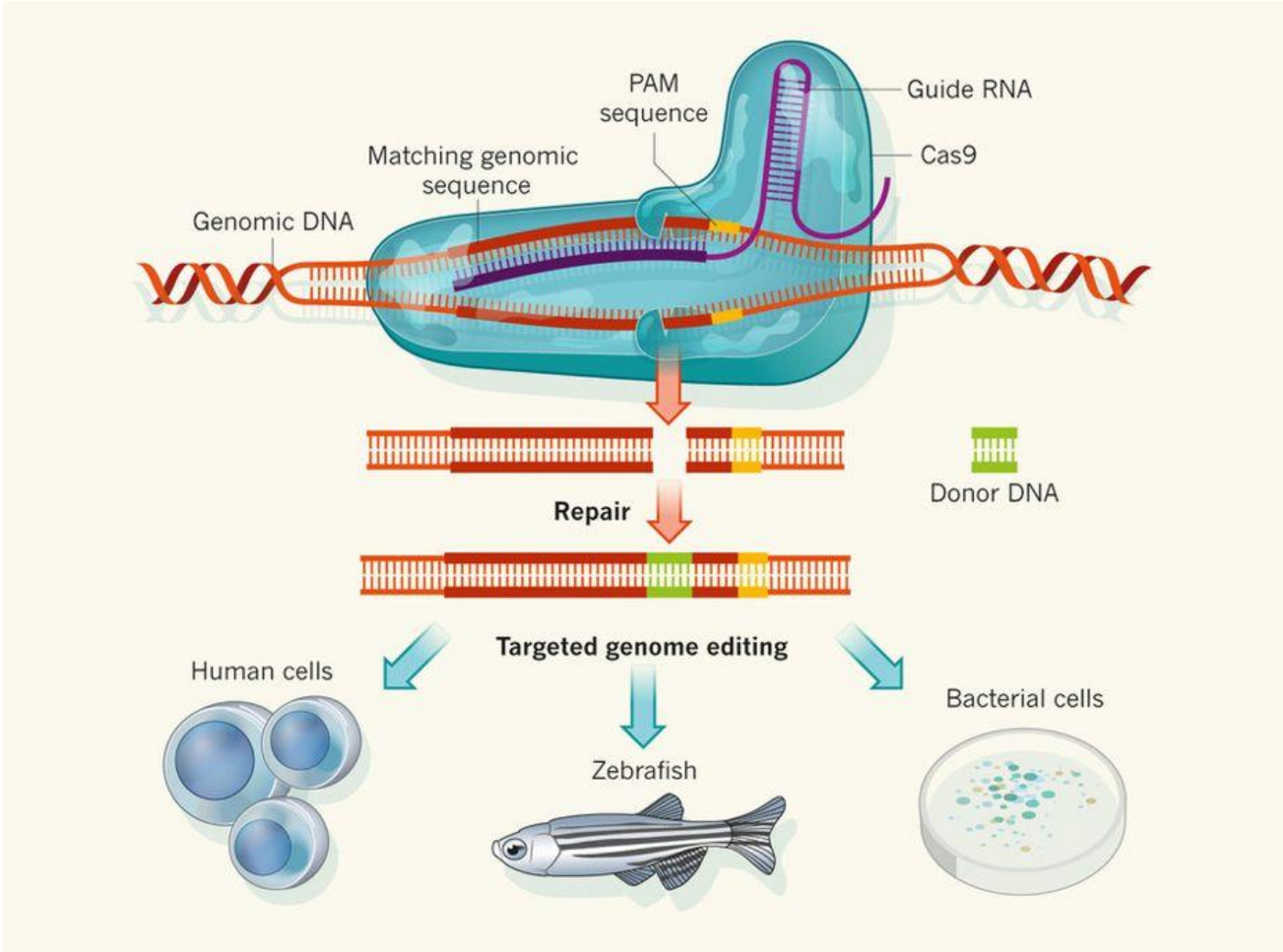
CRISPR- **as**sociated = **Cas**



https://www.nature.com/articles/495050a

BIOTECHNOLOGY

Bitter fight over CRISPR patent heats up

Unusual battle among academic institutions holds key to gene-editing tool's future use.

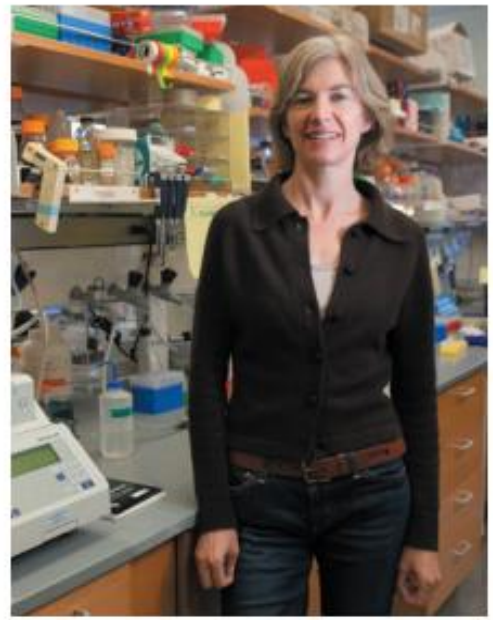
BY HEIDI LEDFORD

A versatile technique for editing genomes has been called the biggest biotechnology advance since the polymerase chain reaction (PCR), and the US Patent and Trademark Office (USPTO) is set to determine who will reap the rewards.

On 11 January, the USPTO granted a request to review a key patent awarded for the technique, known as CRISPR–Cas9. The outcome of the ensuing proceedings, called a patent interference, could be worth millions to the research institutions that are at war over the relevant patents. It might also influence who is allowed to use the technology — and under what terms.

"This is an absolutely humungous biotech patent dispute," says legal scholar Jacob Sherkow of New York Law School. "We're all waiting with bated breath."

CRISPR–Cas9 is a bacterial defence system that uses the enzyme Cas9 to snip DNA at



RYAN ANSON/AP IMAGES FOR HHMI

Jennifer Doudna of the University of California, Berkeley, helped to develop the CRISPR system.

institutions usually come to an agreement to share rights to the invention. "This seems more bitter than disputes I've heard of in the past," she adds.

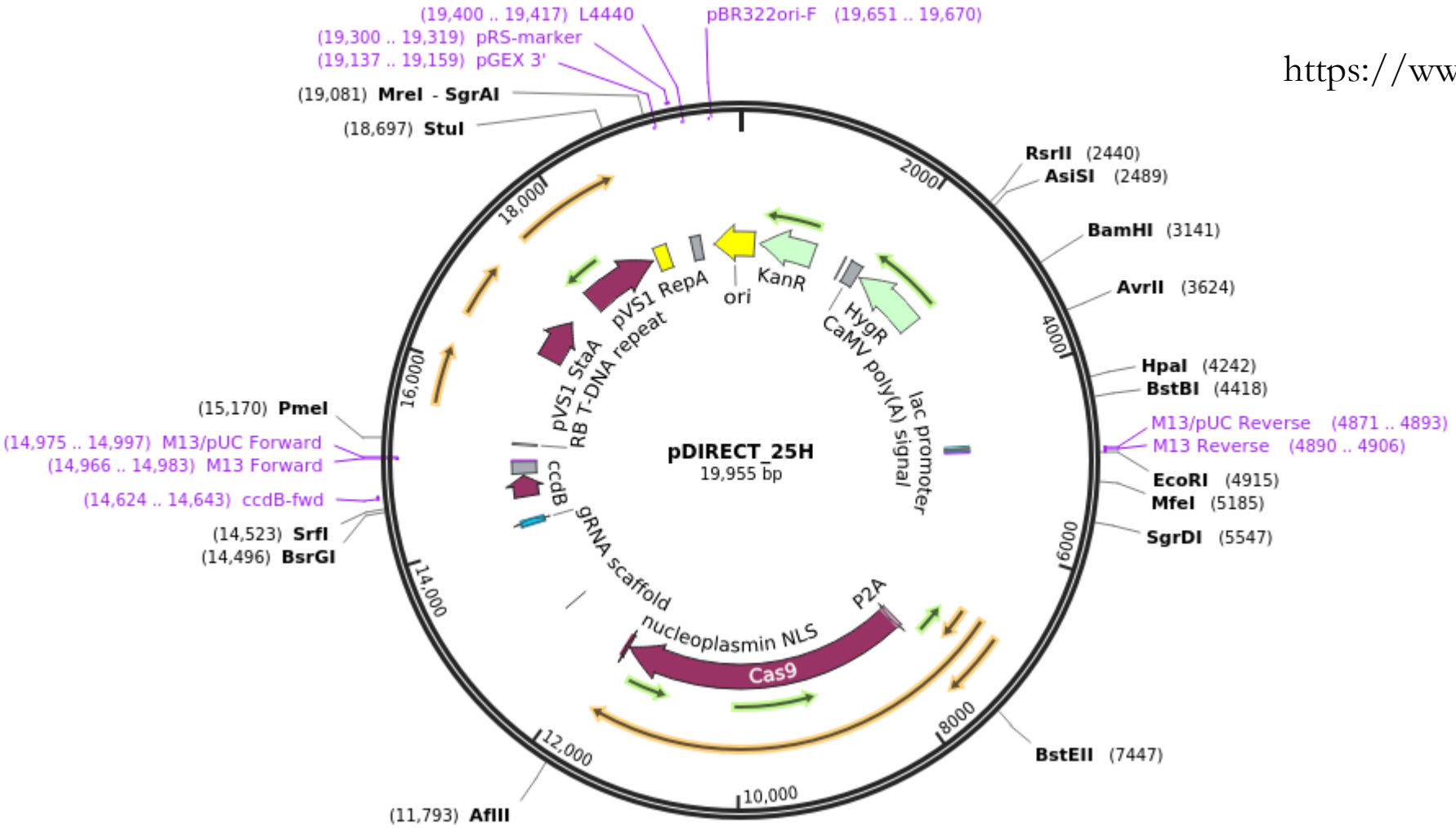
The two patents in question make broad claims to 'foundational' intellectual property thought to be necessary for most lucrative CRISPR–Cas9 applications. But many patents have been filed on CRISPR–Cas9 technologies, and there is still the chance that the winner of the interference will face additional challenges in court. Zhang's group has also reported another enzyme, called Cpf1, that functions much like Cas9. Researchers expect other alternatives to emerge with time.

LICENSING LOOMS

For now, it is unclear how the dispute will affect researchers who use CRISPR–Cas9, if it does so at all. "Patent holders might send out a few cease-and-desist letters, but they probably won't sue academic researchers," says Rodney Sparks, a biotechnology-patent

Exemplo de vetor binário (plasmídeo) para CRISPR/Cas em plantas

https://www.addgene.org/91145/



CRISPR

MENU
nature biotechnology

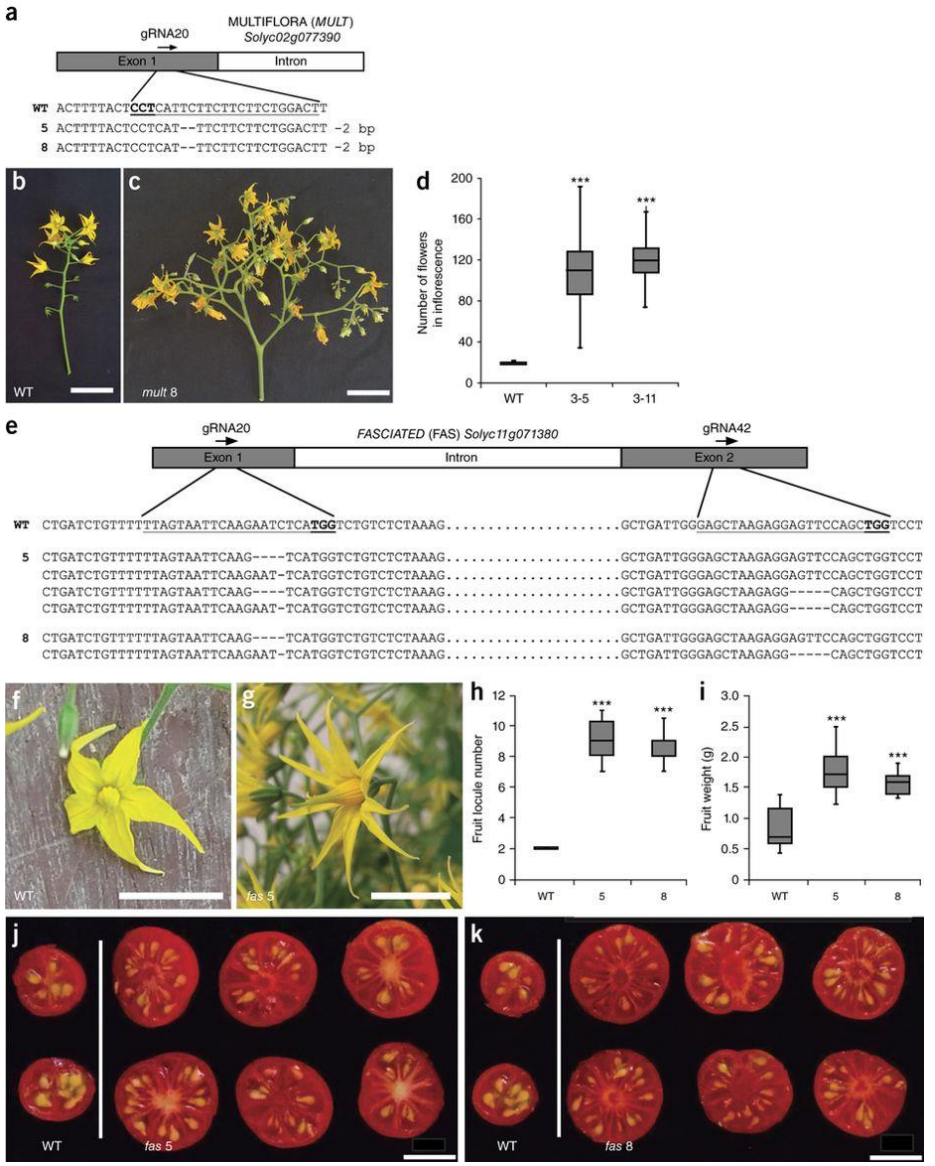
Article | Published: 01 October 2018

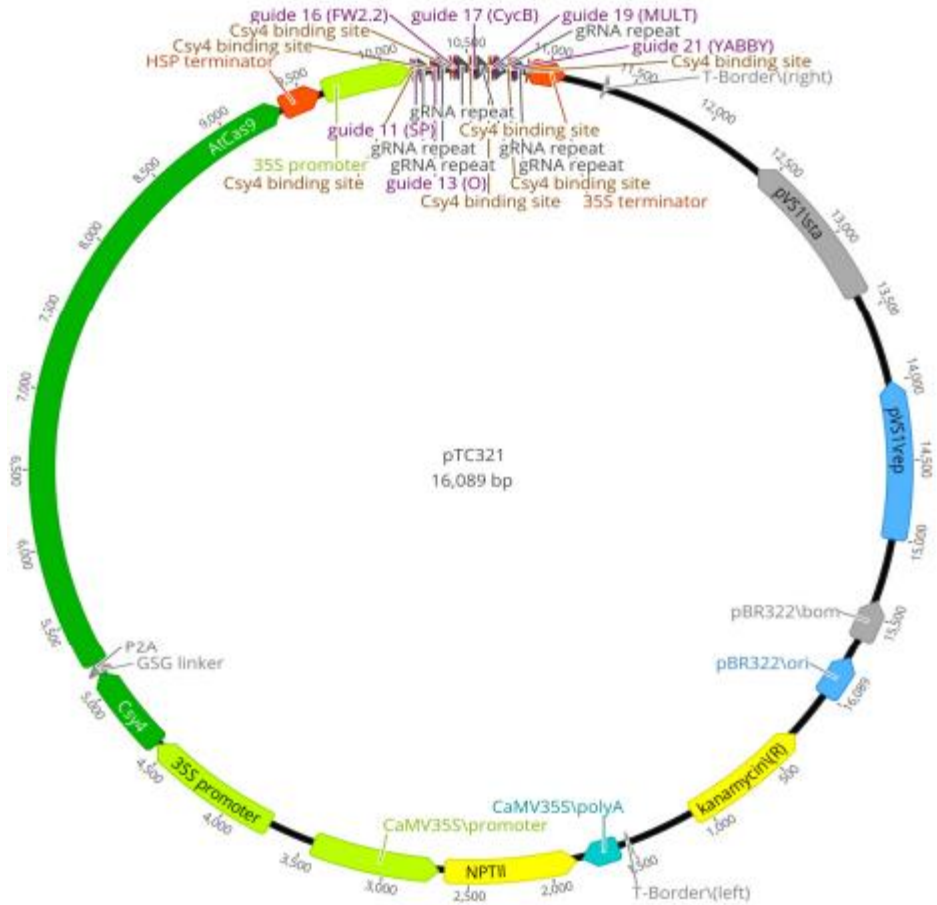
De novo domestication of wild tomato using genome editing

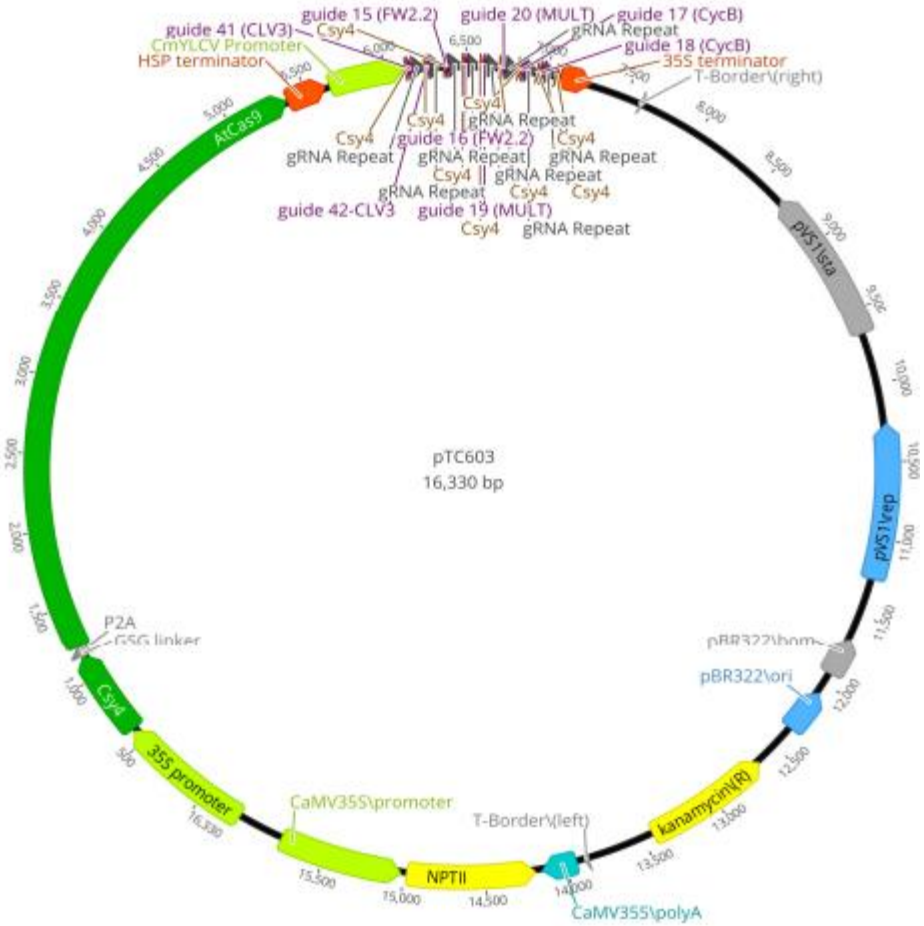
Agustin Zsögön, Tomáš Čermák, Emmanuel Rezende Naves, Marcela Morato Notini, Kai H Edel, Stefan Weinl, Luciano Freschi, Daniel F Voytas, Jörg Kudla & Lázaro Eustáquio Pereira Peres

Nature Biotechnology **36**, 1211–1216 (2018) | Download Citation

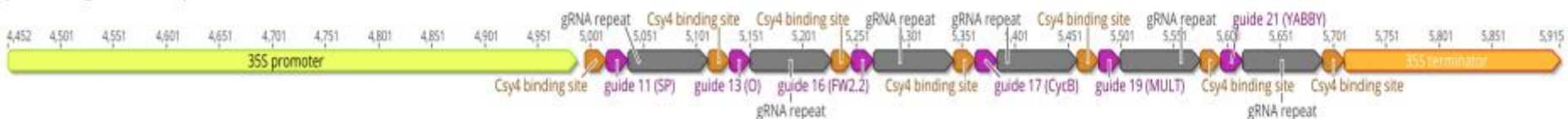
Em tomato, pelo menos 6 loci são chaves para características de domesticação: hábito de crescimento da planta (*SELF-PRUNING*), forma do fruto (*OVATE*) e tamanho do fruto (*FASCIATED* e *FRUIT WEIGHT 2.2*), número de frutos (*MULTIFLORA*), e qualidade nutricional (*LYCOPENE BETA CYCLASE*).



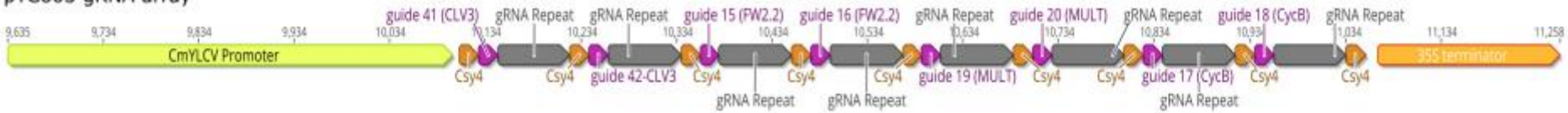




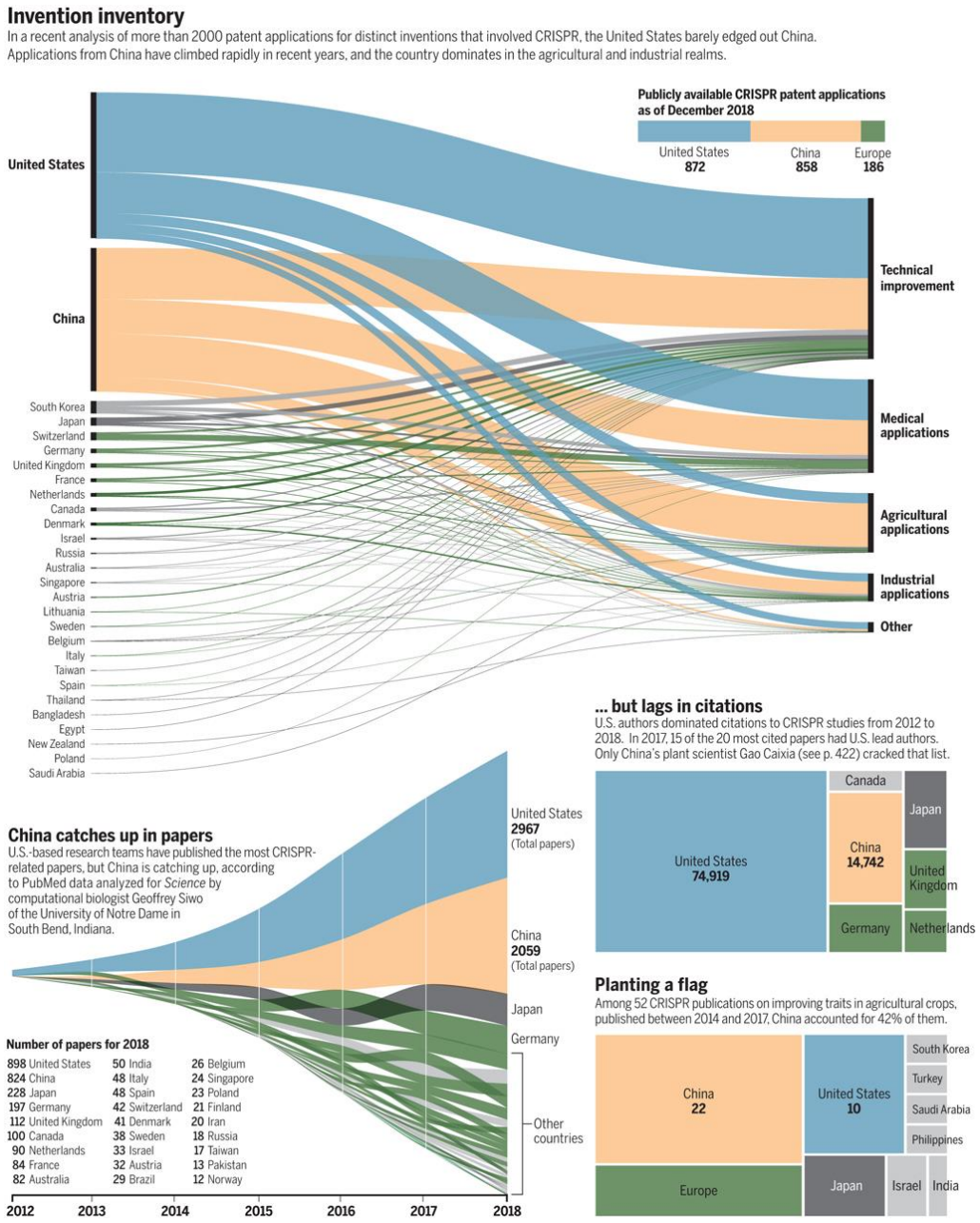
pTC321 gRNA array



pTC603 gRNA array

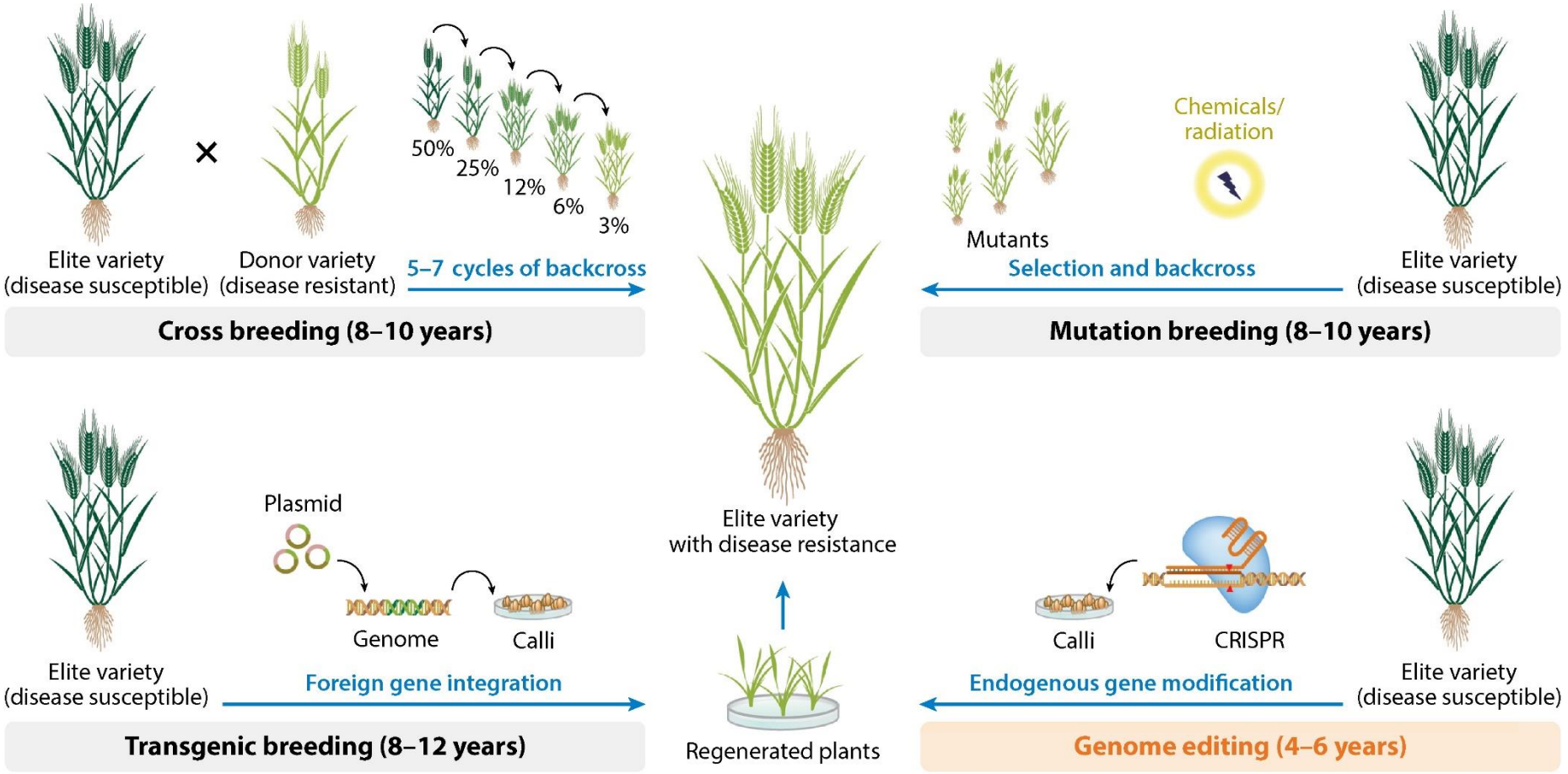


CRISPR na China



02/08/2019

Comparação de Métodos de Melhoramento



Chen K, et al. 2019.
Annu. Rev. Plant Biol. 70:667–97

CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture

Comissão Técnica Nacional de

Biossegurança

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA, INOVAÇÕES E COMUNICAÇÕES

INSTITUCIONAL

A CTNBio

Secretaria Executiva

Processo de OGM

Reuniões

Atas

CTNBio

Resolução Nº 18, de 23 de março de 2018

Republica a Resolução Normativa nº 2, de 27 de novembro de 2006, que "Dispõe sobre a classificação de riscos de Organismos Geneticamente Modificados (OGM) e os níveis de biossegurança a serem...

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Resolução Normativa Nº 16, de 15 de janeiro de 2018

Estabelece os requisitos técnicos para apresentação de consulta à CTNBio sobre as Técnicas Inovadoras de Melhoramento de Precisão A COMISSÃO TÉCNICA NACIONAL DE...

Ler mais »

Técnicas Inovadoras de Melhoramento de Precisão - TIMPs

- Regulações de biossegurança devem se basear em conhecimentos científicos
- Para commodities – regulamentos comuns aceitos por diversos países
- **MAS**, a abordagem regulatória para TIMPs varia entre países
- A edição de genoma utiliza várias abordagens:
  - Deleção ou inserção dirigida, substituição de alelo, conversão de bases...
- Regulação de biossegurança de OGMs é baseada em método e não em produto ou seus riscos potenciais!
- **A introdução de TIMPs, particularmente a edição genômica, trouxe novos desafios regulatórios!!**

Resolução Normativa Nº 16, de 15 de janeiro de 2018

Imprimir

Estabelece os requisitos técnicos para apresentação de consulta à CTNBio sobre as Técnicas Inovadoras de Melhoramento de Precisão

A COMISSÃO TÉCNICA NACIONAL DE BIOSSEGURANÇA – CTNBio, no uso de suas atribuições legais e regulamentares e em observância às disposições contidas nos incisos XV e XVI do art. 14 da Lei nº 11.105, de 24 de março de 2005;

CONSIDERANDO a necessidade de avaliar as Técnicas Inovadoras de Melhoramento de Precisão (TIMP), do inglês Precision Breeding Innovation (PBI) e que também englobam as denominadas Novas Tecnologias de Melhoramento, do inglês New Breeding Technologies -NBTs, à luz dos preceitos previstos na Lei nº 11.105, de 24 de março de 2005;
Considerando que a Lei nº 11.105, de 2005, define moléculas de ADN/ARN recombinante, engenharia genética e organismo geneticamente modificado - OGM nos incisos III, IV e V de seu art. 3º, respectivamente;

Considerando que as TIMP abrangem um conjunto de novas metodologias e abordagens que diferem da estratégia de engenharia genética por transgenia, por resultar na ausência de ADN/ARN recombinante no produto final;

Considerando que as TIMP podem introduzir usos inovadores de ferramentas de biologia molecular, que podem resultar:
1. Na edição precisa de genomas, por indução de mutações específicas, gerando ou modificando alelos selvagens e/ou mutados sem inserção de transgene(s);
2. Em transformação genética e/ou controle de expressão gênica (ativação/inativação);
3. Em regulação epigenética da expressão de genes por mecanismos naturais sem haver modificação genética no indivíduo;
4. Em transformação genética e/ou controle de expressão gênica com genes de espécies sexualmente compatíveis;
5. Em transformação genética temporária e não herdável de células e tecidos;
6. Em infecção permanente ou não no hospedeiro de elementos virais transformados geneticamente;
7. Na criação de alelos com herança autônoma e potencial de recombinação com possibilidade de alterar toda uma população (direcionamento gênico, do inglês: gene drive); e
8. Na construção de genes heterólogos ou novas cópias de genes homólogos.
Resolve:

Art. 1º São considerados exemplos de Técnicas Inovadoras de Melhoramento de Precisão (TIMP), mas não limitadas a estas, as tecnologias descritas no Anexo I integrante desta Resolução Normativa, que podem originar um produto não considerado como um Organismo Geneticamente Modificado (OGM) e seus derivados, conforme definições da Lei nº 11.105, de 24 de março de 2005.
§ 1º O produto a que se refere o caput deste artigo é definido como a descendência, linhagem ou o produto final de um processo que utiliza Técnicas Inovadoras de Melhoramento de Precisão em uma de suas fases de desenvolvimento.
§ 2º Os casos a serem enquadrados não se limitam às tecnologias descritas no Anexo I, uma vez que o avanço rápido e contínuo de diferentes tecnologias poderá propiciar novos produtos, aos quais os preceitos desta Resolução Normativa serão igualmente aplicáveis.
§ 3º Os produtos a que se refere o caput desse artigo implicam em, pelo menos, uma das seguintes características:
I – produto com ausência comprovada de ADN/ARN recombinante, obtido por técnica que emprega OGM como parental;
II – produto obtido por técnica que usa ADN/ARN que não se multiplicará em célula viva;
III – produto obtido por técnica que introduz mutações sítio dirigidas, gerando ganho ou perda de função gênica, com a ausência comprovada de ADN/ARN recombinante no produto;
IV – produto obtido por técnica onde existe a expressão, temporária ou permanente, de moléculas de ADN/ARN recombinante, sem que haja a presença ou introgressão dessas moléculas no produto; e
V – produto onde são utilizadas técnicas que empregam moléculas de ADN/ARN que, absorvidas ou não de forma sistêmica, não causam modificação permanente do genoma.
Parágrafo único. No caso de um produto obtido a partir de um OGM com parecer favorável da CTNBio para liberação comercial, as condições descritas serão aplicáveis somente à característica introduzida por TIMP.

Resolução Normativa Nº 16, de 15 de janeiro de 2018

Imprimir

Estabelece os requisitos técnicos para apresentação de consulta à CTNBio sobre as Técnicas Inovadoras de Melhoramento de Precisão

A COMISSÃO TÉCNICA NACIO

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Art. 1º São considerados exemplos de Técnicas Inovadoras de Melhoramento de Precisão (TIMP), mas não limitadas a estas, as tecnologias descritas no Anexo I integrante desta Resolução Normativa, que podem originar um produto não considerado como um Organismo Geneticamente Modificado (OGM) e seus derivados, conforme definições da Lei nº 11.105, de 24 de março de 2005.

§ 1º O produto a que se refere o caput deste artigo é definido como a descendência, linhagem ou o produto final de um processo que utiliza Técnicas Inovadoras de Melhoramento de Precisão em uma de suas fases de desenvolvimento.

§ 2º Os casos a serem enquadrados não se limitam às tecnologias descritas no Anexo I, uma vez que o avanço rápido e contínuo de diferentes tecnologias poderá propiciar novos produtos, aos quais os preceitos desta Resolução Normativa serão igualmente aplicáveis.

§ 3º Os produtos a que se refere o caput desse artigo implicam em, pelo menos, uma das seguintes características:

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III – produto obtido por técnica que introduz mutações sítio dirigidas, gerando ganho ou perda de função gênica, com a ausência comprovada de ADN/ARN recombinante no produto;

IV – produto obtido por técnica onde existe a expressão, temporária ou permanente, de moléculas de ADN/ARN recombinante, sem que haja a presença ou introgressão dessas moléculas no produto; e

V – produto onde são utilizadas técnicas que empregam moléculas de ADN/ARN que, absorvidas ou não de forma sistêmica, não causam modificação permanente do genoma.

Parágrafo único. No caso de um produto obtido a partir de um OGM com parecer favorável da CTNBio para liberação comercial, as condições descritas serão aplicáveis somente à característica introduzida por TIMP.

TIMPS – ANEXO I

- ...Art. 1º São considerados **exemplos de Técnicas Inovadoras de Melhoramento de Precisão (TIMP),** mas não limitadas a estas, as tecnologias descritas no Anexo I integrante desta Resolução Normativa, que podem originar um produto não considerado como um Organismo Geneticamente Modificado (OGM) e seus derivados, conforme definições da Lei nº 11.105, de 24 de março de 2005...

...

- 5. **TÉCNICA: Mutagênese Sítio Dirigida**.
- 5.1 RESUMO DA TÉCNICA: Complexos proteicos ou riboproteicos capazes de causar mutagênese sítio dirigida em microrganismos, plantas, animais e células humanas....

...

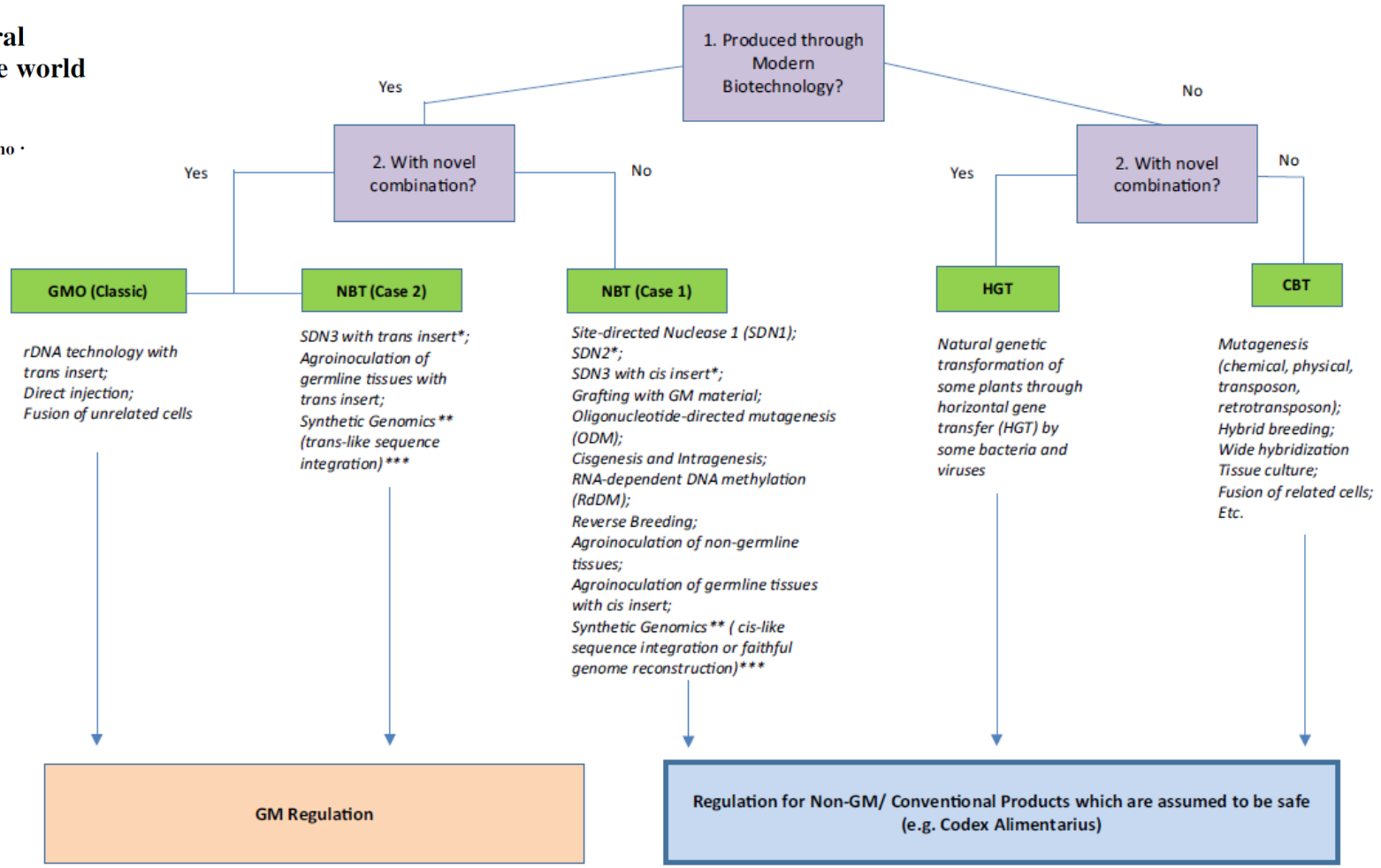
- 8. **TÉCNICA**: **RNAi uso tópico/sistêmico.**
- 8.1 RESUMO DA TÉCNICA: Uso de RNA fita dupla ("dsRNA") com sequência homóloga ao(s) gene(s) alvo para silenciamento específico desse(s) gene(s). As moléculas engenheiradas de dsRNA podem ser introduzidas/absorvidas pela célula a partir do ambiente....

Transgenic Res (2021) 30:551–584
https://doi.org/10.1007/s11248-021-00257-8

GENOME EDITING IN PLANTS

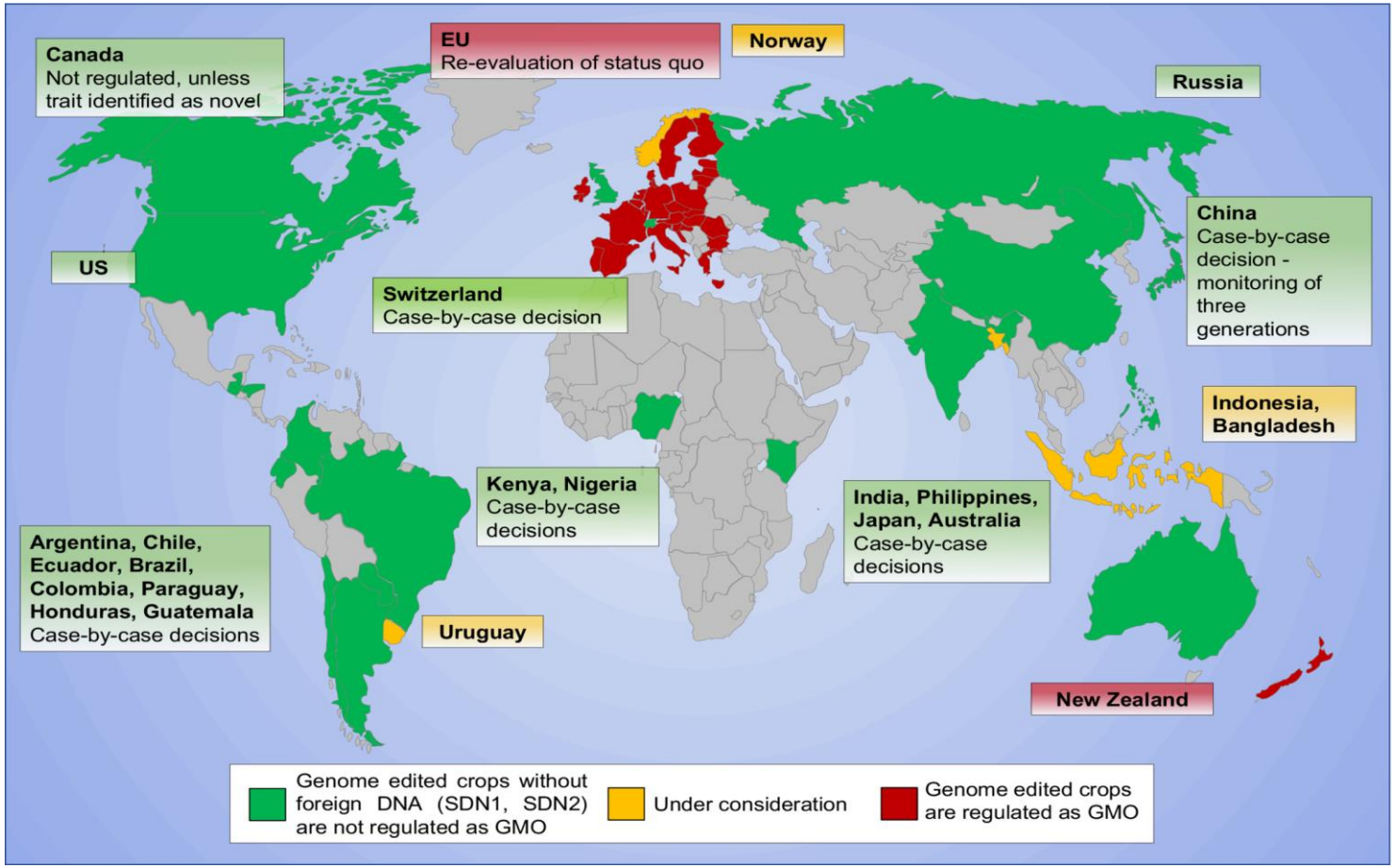
Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world

Jon Entine · Maria Sueli S. Felipe · Jan-Hendrik Groenewald · Drew L. Kershen · Martin Lema · Alan McHughen · Alexandre Lima Nepomuceno · Ryo Ohsawa · Reynante L. Ordonio · Wayne A. Parrott · Hector Quemada · Carl Ramage · Inez Slamet-Loedin · Stuart J. Smyth · Diane Wray-Cahen



*Includes insertion using the new CRISPR-CAS with Prime Editing (Anzalone et al, 2019)
** Not to be confused with Synthetic Biology, which specializes on sequences/genetic elements (e.g. unnatural base pairs) in the genome that are not found in nature (beyond novel combination).
***Pertains to a largely synthetic assembled genome.

Regulação de edição de genoma para uso comercial



New Phytologist, First published: 23 June 2022, DOI: (10.1111/nph.18333)

CRISPR – liberação Europa

nature International weekly journal of science 22/02/17

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Archive > Volume 542 > Issue 7642 > Editorial > Article

NATURE | EDITORIAL

Gene editing in legal limbo in Europe

The European Union is dragging its feet on gene-editing rules and scientists should push the issue.

22 February 2017

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NEWS · 19 JANUARY 2018

European court suggests relaxed gene-editing rules

Judicial opinion says restrictive regulations may not apply to plants and animals bred using CRISPR technique.

THIS WEEK EDITORIALS

Legal limbo

Europe is dragging its feet on gene-editing rules and scientists should push the issue.

Germany is having trouble deciding whether plants that are gene-edited should be regulated as if they were genetically modified (GM). Confused? You're not alone: the issue has split the German government and has left scientists across Europe in limbo.

Plant scientists say that new editing tools, including CRISPR–Cas9, involve no more than making tiny, precisely targeted changes to a gene that are indistinguishable from natural mutations. But opponents say that any form of meddling with genes is potentially perilous.

Germans attach great value to public dialogue. So on 14 February, the Leopoldina, Germany's national science academy, hosted a debate on the issue. Officials from the federal environment ministry and its office for nature protection spoke passionately in favour of ever-greater regulation, whereas the agriculture ministry and the office for consumer protection and food safety disagreed.

The debate might never have taken place if the European Union itself had been able to decide on the issue. But it is habitually paralysed whenever genetic modification is discussed. Two years ago the European Commission requested all member states to hold back on giving the all-clear on gene editing while it considered its options. Now its hand is being forced, ever so slowly, by the referral of the issue by France to the European Court of Justice (ECJ) last October. French non-governmental organizations and trade unions had called on the French state to regulate organisms created through all methods of mutagenesis, including classical methods. They argued that easy-to-use, modern gene-editing tools will encourage large numbers of new plants to be created whose environmental impacts are uncertain. At the Leopoldina meeting, the German office for nature protection aligned itself with this argument.

The ECJ told *Nature* that a decision is not expected before 2018 because the case is so politically sensitive. That's a long time to wait, given that so much is at stake. GM-style regulation is complex and exorbitantly costly. CRISPR technology, although very new, has already led to many gene-edited plants that are ready for outdoor field trials. Such studies should not be held up. Some are intended to shed light on basic plant biology, such as how plants adapt themselves so readily to their environments. Others will determine whether the gene-edited plants have new traits that make them better crops. European scientists are competing with countries such as the United States, where gene-edited products are not considered equivalent to GM products, at least for now. And earlier this month the European Ombudsman stated that the legal limbo does not mean that gene editing should be put on freeze.

Some EU member states are forging their own way through the muddle. In 2015, Sweden decided that the technical and legal issues in favour of non-regulation were crystal clear and told its plant scientists that they could go ahead. It has promised to reverse its position should the EU decide on regulation. Stefan Jansson at Umeå University made such swift progress that he hosted a press lunch last summer where he served up 'tagliatelle with CRISPRy fried vegetables' using ingredients from his garden, including a gene-edited cabbage. According to those present, it was delicious. Last year, Finland chose a similar path, although no field trials have begun.

"CRISPR technology has already led to many gene-edited plants that are ready for outdoor field trials."

Sabres are rattling in the Netherlands, where the parliament's lower house called on the government last week to consider the exclusion of most forms of gene editing from GM regulation. The United Kingdom has maintained silence, and will in any case be under no obligation to follow EU rules once Brexit is complete.

Germany, meanwhile, is being forced to wait for the ECJ decision. In 2015, the consumer protection office told the San Diego-based biotechnology company Cibus that its herbicide-resistant oilseed rape, created using one of the earlier gene-editing technologies, would not need to be regulated in the country. Opponents immediately brought a court case — but that local court is now awaiting ECJ guidance. And during this election year, the German government is highly unlikely to risk making sensitive decisions.

The ECJ has an unfortunate history of delivering highly conservative or scientifically confused verdicts on complex biological issues. In 2011, it outlawed patents that depended even indirectly on human embryonic stem-cell lines, adding that similar basic research was immoral. And in the same year it nearly upended the European honey market with a muddled decision about alleged traces of pollen from GM maize.

Plant scientists should spend the waiting time engaging in public dialogue like the one Germany is leading about the safety and value of gene editing. Reason and science need to prevail this time. ■

CRISPR – liberação Europa

IN FOCUS NEWS

BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency's regulatory process — making it the first CRISPR-edited organism to receive a green light from the US government.

"The research community will be very happy with the news," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yinong Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (*Agaricus bisporus*) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a handful of base pairs in the mushroom's genome, Yang knocked out one of six PPO genes — reducing the enzyme's activity by 30%.

STUART MCCALL/GETTY

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

AGENCY RULES

The mushroom is one of about 30 genetically modified organisms (GMOs) to sidestep the USDA's regulatory system in the past 5 years. In each case, the agency's Animal and Plant Health Inspection Service (APHIS) has said that the organisms — mostly plants — do not qualify as something that the agency must regulate. (Once a crop passes the USDA reviews, it may still undergo a voluntary review by the US Food and Drug Administration.)

Several of the plants that bypassed the USDA were made using gene-editing techniques such as the zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) systems. But until now, it was not clear whether the USDA would give the same pass to organisms engineered with science's hottest tool, CRISPR–Cas9.

Yang first presented the crop to a small group of USDA regulators in October 2015, after being encouraged to do so by an APHIS official. "They were very excited," Yang says: "There was certainly interest and a positive feeling" at the meetings. He followed up with an official letter of enquiry to the agency later that month.

The USDA's answer came this week. "APHIS does not consider CRISPR/Cas9-edited white button mushrooms as described in your October 30, 2015 letter to be regulated," the agency wrote to Yang on 13 April.

> ***"I am confident we'll see more gene-edited crops falling outside of regulatory authority."***

Yang's mushroom did not trigger USDA oversight because it does not contain foreign DNA from 'plant pests' such as viruses or bacteria. Such organisms were necessary for genetically modifying plants and fungi in the 1980s and 1990s, when the US government developed its framework for regulating GMOs. But newer gene-editing techniques that do not involve plant pests are quickly supplanting the old tools.

The United States is revamping its rules for regulating GMOs, which collectively are known as the Coordinated Framework for Regulation of Biotechnology. To that end, the US National Academies of Sciences, Engineering and Medicine have convened a committee that is charged with predicting what advances will be made in biotechnology products over the next five to ten years. It will hold its first meeting on 18 April.

In the meantime, Yang is mulling over whether to start a company to commercialize his modified mushroom. Fruits and vegetables that resist browning are valuable because they keep their colour longer when sliced, which lengthens their shelf life. In the past 18 months, biotech companies have commercialized genetically engineered non-browning apples and potatoes.

"I need to talk to my dean about that. We'll have to see what the university wants to do next," says Yang about the prospect of bringing his mushroom to market. But he notes that in September 2015, Penn State filed a provisional patent application on the technology. ■

21/04/2016

NEWS IN FOCUS

CHRIS RATCLIFFE/BLOOMBERG/GETTY

In the EU, gene-edited crops and food will be treated in the same way as genetically modified organisms.

GENE EDITING

EU law deals blow to CRISPR crops

Top court's ruling threatens research on gene-edited plants.

BY EWEN CALLAWAY

Gene-edited crops should be subject to the same stringent regulations that govern conventional genetically modified (GM) organisms, Europe's highest court ruled on 25 July.

The decision, handed down by the Court of Justice of the European Union (ECJ) in Luxembourg, is a major setback for proponents of gene-edited crops, including many scientists. They had hoped that organisms created using relatively new, precise gene-editing technologies such as CRISPR–Cas9 would be exempted from existing European law, which has limited the planting and sale of GM crops.

Instead, the ECJ ruled that crops created using these technologies are subject to a 2001 directive. That law was developed for older breeding techniques, and it puts high hurdles in the way of developing GM crops for food.

"It is an important judgment, and it's a very rigid judgment," says Kai Purnhagen, a legal scholar at Wageningen University and Research in the Netherlands who specializes in European and international law. "It means for all the new inventions, such as CRISPR-Cas9 food, you would need to go through the lengthy approval process of the European Union."

That is likely to hinder investment in crop research using these tools in the EU, says Purnhagen. "From a practical perspective, I don't think this will be at all of interest for business. So they will move somewhere else," he says.

The ruling is "tremendously disappointing", says Nigel Halford, a crop geneticist at Rothamsted Research in Harpenden, UK. "It's a real hit to the head," he says. Gene-editing techniques will still be used as a research tool for developing crops, he adds, but he doubts that companies in Europe will have much appetite to develop them. "They are not going to invest in a technology they see not having any commercial application," Halford says.

Environmental organization Friends of the Earth in Amsterdam, meanwhile, applauded the court's decision in a statement. It also called for all products made through gene editing to be regulated, assessed for their health and environmental impacts, and labelled.

DNA CHANGES

The 2001 EU directive behind the ECJ's decision concerns the intentional release of GM organisms into the environment — and was aimed at species into which entire genes, or long stretches of DNA, had been inserted. The law exempts organisms whose genomes were modified using 'mutagenesis' techniques, such as irradiation, which introduce changes to an organism's DNA but don't add foreign genetic material.

In 2016, the French government asked the ECJ to interpret the directive in light of plant-breeding techniques that have since emerged.

Many plant breeders and scientists contend that gene-editing techniques such as CRISPR–Cas9 should be considered mutagenesis, just like irradiation, and thus be exempt from the directive, because they can involve changes to DNA and not the insertion of foreign genes. But people opposed to GM organisms contend that the deliberate nature of alterations made through gene editing means that they should fall under the directive.

In January, an advocate-general with the court, Michal Bobek, issued a 15,000-word opinion that both sides claimed was partly in their favour. He said that gene-edited crops do constitute GM organisms under the original directive, but also that species modified using technologies discovered since 2001 — such as those used for gene editing — could be exempted, as long as they don't contain DNA from other species, or artificial DNA.

But in its ruling, the ECJ determined that only mutagenesis techniques that have "conventionally been used in a number of applications and have a long safety record are exempt from those obligations". Organisms made using mutagenesis techniques developed after 2001 — including gene editing — are not exempt from the directive.

NO INCENTIVE

"This will have a chilling effect on research, in the same way that GMO legislation has had a chilling effect for 15 years now," says Stefan Jansson, a plant physiologist at Umeå University in Sweden. Gene-edited crops will not vanish from European research labs, but he worries that the funding to develop them could dry up. "If we cannot produce things that society finds helpful, then they will be less likely to fund us."

Jansson also has practical concerns about the ruling. He developed a 'CRISPR cabbage' that he has consumed, and which was growing in his home garden as he spoke to *Nature*. "I took a photo yesterday, and I took another after the ruling. It's still the same plant. Yesterday it wasn't a GMO, and now it's a GMO. I'm a bit curious what I have to do. Do I have to remove it?"

Purnhagen says that the ruling leaves open a possible loophole, whereby if scientists can prove that gene-editing techniques are as safe as mutagenesis methods already exempt from the law, such as irradiation, the new techniques, too, could earn an exemption.

But he doubts that researchers and businesses developing gene-edited crops will hold out hope. "I can't see CRISPR–Cas9 and all these new technologies will be profitable in the European Union. I can't see this happening. I think this research will move somewhere else." ■

02/08/2018

GABA-enriched tomato is first CRISPR-edited food to enter market

Sanatech Seed's Sicilian Rouge CRISPR-edited 'health-promoting' tomatoes reach consumers and may open the market to more genome-edited fruit, vegetables and even fish.

Genome-edited food made with CRISPR–Cas9 technology is being sold on the open market for the first time. Since September, the 'Sicilian Rouge' tomatoes, which are genetically edited to contain high amounts of γ-aminobutyric acid (GABA), have been sold direct to consumers in Japan by Tokyo-based Sanatech Seed. The company claims oral intake of GABA can help support lower blood pressure and promote relaxation.

In Japan, dietary supplements and foods enriched for GABA are popular among the public, says Hiroshi Ezura, chief technology officer at Sanatech and a plant molecular biologist at the University of Tsukuba. "GABA is a famous health-promoting compound in Japan. It's like vitamin C," he says. More than 400 GABA-enriched food and beverage products, such as chocolates, are already on the Japanese market, he says. "That's why we chose this as our first target for our genome editing technology," he says.

Sanatech, a startup from the University of Tsukuba, first tested the appetite of consumers in Japan for the genome-edited fruit in May 2021 when it sent free seedling CRISPR-edited tomato plants to about 4,200 home gardeners who had requested them. Encouraged by the positive demand, the company started direct internet sales of fresh tomatoes in September and a month later took orders for seedlings for next growing season. Japan's regulators approved the tomato in December 2020.

Since its inception a decade ago, CRISPR–Cas9 genome editing has become a tool of choice for plant bioengineers. Researchers have successfully used it to develop non-browning mushrooms, drought-tolerant soybeans and a host of other creative traits in plants. Many have received a green light from US regulators. But before Sanatech's tomato, no CRISPR-edited food crops were known to have been commercialized.

Consumers may find food ingredients made with some of the older DNA editing techniques, such as transcription activator-like effector nucleases (TALENs). Indeed, Calyxt in 2019 commercialized a TALEN-edited soybean oil that is free of *trans* fats. Genome editing tools have also been used to transform a host of ornamental plants. So it was only a matter of time before a CRISPR-edited crop reached palates.

A CRISPR-edited tomato containing higher GABA than its unedited counterparts takes off in Japan. Credit: Aflo Co., Ltd. / Alamy Stock Photo

More interesting, however, is that the developer chose this high GABA trait as a first target. GABA is an amino acid and a neurotransmitter that blocks impulses between nerve cells in the brain. The molecule is found natively in the human body and is also ubiquitously present in plants, animals and microorganisms, as well as in food. It can be synthesized by fermenting food and has been developed as a nutritional supplement in some regions.

Sanatech's researchers increased the amount of GABA in tomato by manipulating a metabolic pathway called the GABA shunt. There, they disabled a gene that encodes calmodulin-binding domain (CaMBD). Removal of CaMBD enables increased activity of the enzyme glutamic acid decarboxylase, which catalyzes the decarboxylation of glutamate to GABA, thus raising levels of the molecule.

Sanatech has been careful not to claim that its tomatoes therapeutically lower blood pressure and promote relaxation. Instead, the company implies it, by advertising that consuming GABA, generally, can achieve these effects and that its tomatoes contain high levels of GABA. This has raised some eyebrows in the research community, given the paucity of evidence supporting GABA as a health supplement.

To support the blood-pressure assertion, Sanatech cites two human studies: a 2003 paper on the effect of consuming fermented milk containing GABA and a 2009 paper of the effects of GABA, vinegar and dried bonito. Both studies were conducted in people with mild hypertension and showed blood-pressure-lowering effects.

But the papers lack good control groups, and the effects in the experimental groups could be explained by factors other than GABA, says Maarten Jongsma, a molecular cell biologist at Wageningen University & Research in the Netherlands, who studies the effects of plant compounds on human nutrition. "There's no consensus" on the health benefits of consuming GABA, nor evidence that it can cross the blood–brain barrier and reach the central nervous system, adds Renger Witkamp, a nutrition scientist also at Wageningen.

To support the claim that GABA promotes relaxation, Sanatech points to six studies in humans that examined the effect of orally consumed GABA on stress, mood, fatigue or sleep. But a systematic review published in 2020 that examined all six of these papers plus eight more on the topic came to a different conclusion. The authors, who hailed from Japan, Australia and the United Kingdom, summarized: "There is limited evidence for stress and very limited evidence for sleep benefits of oral GABA intake."

Sanatech's tomatoes, called the Sicilian Rouge High GABA, contain about four to five times more GABA than their conventional counterpart, Ezura says. Whether that will lower blood pressure any more than eating regular tomatoes is unclear. Sanatech has not performed this kind of intervention study, although it plans to do so, Ezura says. The company is working to complete an additional notification with the Japanese government on the health benefit claim.

Sanatech's marketing strategy has been to target consumers directly and generate positive buzz among home gardeners. The company created an online platform for gardeners to swap tips. It also held a contest to see which home gardener could grow tomatoes with the highest amount of GABA. (The winning tomato had 20 times more GABA than conventional tomatoes.)

That's a smart marketing strategy for genome-edited fruit and vegetables, especially those with boutique traits, says Cathie Martin, a plant scientist at the John Innes Centre in Norwich, UK. "You find a group of people who feel as though they have some ownership of the product," she says. You then help build up a community of people who want to grow and eat the vegetable, and this launches the product on a positive track, she says.

Martin is the creator of the 'purple tomato', a variety that is genetically modified to contain higher levels of the anti-inflammatory compound anthocyanin, which she debuted in 2008 in these pages. Over the past 14 years, without the resources of a large company, she and an "un-financed, dedicated band of enthusiasts" have been trying to push the product to market on their own, she says.

Her challenge of commercializing a bioengineered crop is one that most small plant biotech companies have also faced, particularly those developing boutique varieties. "The regulatory cost is so high that there are very few traits that you could actually even consider engineering in a crop like tomato," says James Giovannoni, a plant molecular biologist at the Agricultural Research Service at the US Department of Agriculture (USDA). That's why, since the mid-1990s, most commercial efforts in the genetic engineering of plants have focused on high-dollar crops, such as soybean, corn (maize), wheat, canola and cotton, with traits that make farmer's jobs easier and their harvests more profitable.

Meanwhile, nutritionally enhanced crops have been stillborn. The few examples on the market include soybeans and canola with modified oil and fatty acid content, and nutritionally improved corn for animal feed. Scores more, such as the high β-carotene super-banana, have been developed but sit in limbo on laboratory shelves. The storied 'golden rice', which is enhanced with provitamin A and has been in limbo for 20 years, just a few months ago received approval in the Philippines for commercial cultivation.

So Sanatech's high-GABA tomato, as a nutritionally enhanced crop, stands out. The fact that it was engineered using CRISPR seems to help with consumer acceptance, especially as such crops aren't being called "GMOs," or "genetically modified organisms." Instead, they're dubbed "genome-edited." This change in nomenclature alone seems to have quelled a lot of the backlash historically launched against bioengineered plants.

Some regulators are making a distinction between the old and new technologies too. The USDA has repeatedly ruled that genome-edited crops fall outside of its purview. Plant biotechnologists who submit such inquiries through the agency's "Am I Regulated?" process typically get a response within a few months and receive a green light to grow their genome-edited plants without further oversight.

This has reduced the US regulatory burden for genome-edited plants to next to nothing. Brazil, Argentina and Australia have taken a similar approach. China has established a regulatory process for genome-edited agricultural organisms, although none has yet been approved, says Hongliang Zhu, a professor at China Agricultural University in Beijing, speaking on behalf of himself and not his employer or government. Europe has essentially banned genome-edited foods, lumping them in with first-generation GMOs, although there have been calls to rethink the policy.

Many other countries still lack any policy on the technology, slowing commercial

Japan embraces CRISPR-edited fish

Japan has developed a fleshier red sea bream with genome editing. Moonie's World Photography / Alamy Stock Photo.

Japan has approved the sale of two CRISPR-edited fish: a tiger puffer and a red sea bream, both developed by the Kyoto-based startup Regional Fish Institute with Kyoto University and Kindai University. The fish are engineered to grow bigger than their conventional counterparts. Researchers achieved the trait in tiger puffer by disrupting the leptin receptor gene, which controls appetite, causing the fish to eat more and increasing the speed at which they gain weight. The edited fish grow 1.9 times heavier than conventional tiger puffers, allowing them to reach market size sooner, according to the company. For red sea bream, the researchers disabled the protein myostatin, which suppresses muscle growth, allowing the fish to grow about 1.2 times larger on the same amount of food. The traits are expected to reduce production costs of farming the fish, which will be grown in tanks on land. Regional Fish started a crowdfunding campaign to finance the commercialization of its products. Japan regulates genome-edited food through two agencies: the Ministry of Health, Labour and Welfare and the Ministry of Agriculture, Forestry and Fisheries. The approvals for the tiger puffer and red sea bream bring the total number of approved CRISPR-edited foods in Japan to three. The ministries in December 2020 approved a CRISPR-edited tomato that has been engineered to have increased levels of γ-aminobutyric acid (GABA) for its perceived health benefits. The developer of the tomato, Tokyo-based Sanatech Seed, began selling the tomatoes in September.

Published online: 30 December 2021
https://doi.org/10.1038/s41587-021-01197-8

TIMPS – ANEXO I

- ...Art. 1º São considerados **exemplos de Técnicas Inovadoras de Melhoramento de Precisão (TIMP),** mas não limitadas a estas, as tecnologias descritas no Anexo I integrante desta Resolução Normativa, que podem originar um produto não considerado como um Organismo Geneticamente Modificado (OGM) e seus derivados, conforme definições da Lei nº 11.105, de 24 de março de 2005...

...

- 5. **TÉCNICA: Mutagênese Sítio Dirigida**.
- 5.1 RESUMO DA TÉCNICA: Complexos proteicos ou riboproteicos capazes de causar mutagênese sítio dirigida em microrganismos, plantas, animais e células humanas....

...

- 8. **TÉCNICA**: **RNAi uso tópico/sistêmico.**
- 8.1 RESUMO DA TÉCNICA: Uso de RNA fita dupla ("dsRNA") com sequência homóloga ao(s) gene(s) alvo para silenciamento específico desse(s) gene(s). As moléculas engenheiradas de dsRNA podem ser introduzidas/absorvidas pela célula a partir do ambiente....

RNA de interferência (RNAi)

Histórico - RNA antissenso

Table 1 A summary of commercial developments in agbiotech developed with RNA-mediated gene suppression.

Company	Crop	Trait gene	RNA-based gene suppression approach	Regulatory approval (animal feed, human food and/or environmental)	Phenotypic description
Calgene (now Monsanto)	Tomato (FLAVR SAVR)	Polygalacturonase	Antisense	US, Canada, Mexico, Japan	Delayed fruit ripening
Zeneca London, UK	Tomato	Polygalacturonase	Antisense and co-suppression	US, Canada, Mexico	Delayed fruit ripening
DNA Plant Technology	Tomato	Aminocyclopropane cyclase	Co-suppression	US, Canada, Mexico	Delayed fruit ripening
Vector Tobacco Durham, NC	Tobacco	Quinolinic acid phosphor-ibosyltransferase	Antisense	US	Reduced nicotine levels
DuPont Canada Agricultural Products Ontario, Canada	Soybean	Fatty acid desaturase	Co-suppression	US, Canada, Japan, Australia	High oleic acid soybean
Florigene Pty. Ltd.	Carnation	1-aminocyclopropane-1-carboxylic acid	Co-suppression	Australia, European Union	Longer vase life
US Department of Agriculture	Plum	Plum pox virus coat protein	Co-suppression	US	Viral resistance

Data source: Agbios (http://www.agbios.com)

Ch-Ham et al. 2010. Nat Biotech

Flavr Savr (Calgene)

O tomate *Flavr Savr* foi desenvolvido pela Calgene, Davis, Califórnia. **Baseado na inserção de gene da enzima poligalacturonase (degrada componentes da pectina) no sentido anti-senso!!**

O FDA não exigiu aprovação para liberação, no entanto a Calgene submeteu voluntariamente o *Flavr Savr* para aprovação em 1989.

Em **1994**, o Departamento de Agricultura dos Estados Unidos aprovou por que este não apresentava risco ao ambiente.

Histórico

1990s - uso de RNA antisenso

- **1990:** CHS/DFR em petúnias
  - **Cossupressão**....*post-transcriptional inhibition of gene expression*

Flavonoid Genes in Petunia: Addition of a Limited Number of Gene Copies May Lead to a Suppression of Gene Expression

Alexander R. van der Krol,[1] Leon A. Mur, Marcel Beld, Joseph N.M. Mol,[2] and Antoine R. Stuitje
Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

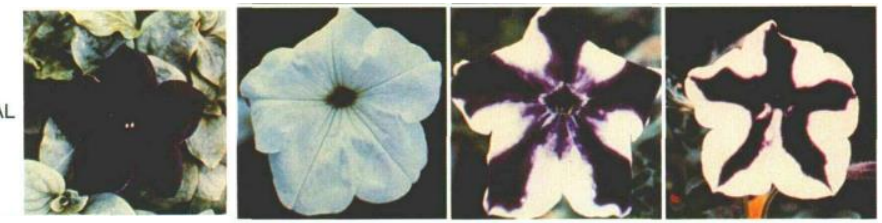
The mechanism of suppression by sense genes may involve interference of RNA strands with the transcription process itself. The transcription process may be blocked

Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes *in trans*

Carolyn Napoli,[1] Christine Lemieux, and Richard Jorgensen[2]
DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, California 94608

nate nature of this interaction and its effect on expression, we have coined the term "co-suppression" to refer to the phenomenon.

PARENTAL

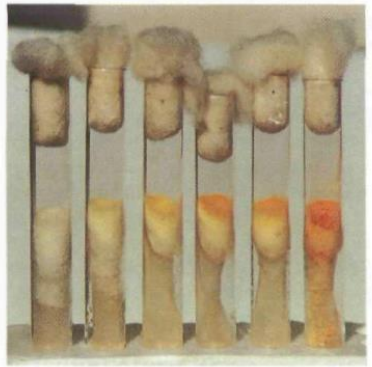


Histórico

- **1992:** *ALB-1* e *ALB-3* em *Neurospora crassa* – não transcritos
  - **"Quelling"**

Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences

Nicoletta Romano and Giuseppe Macino*
Dipartimento di Biopatologia Umana, Sezione di Biologia Cellulare, Policlinico Umberto 1, Università di Roma 'La Sapienza', 00161 Rome, Italy.



Cossupressão em outros organismos

- ***C. elegans:*** *PAR-1* (Guo & Kemphues, 1995)
- ***Drosophila*:** *Adh-1* (Pal-Badhra et al., 1997)

***par-1*, a Gene Required for Establishing Polarity in C. elegans Embryos, Encodes a Putative Ser/Thr Kinase That Is Asymmetrically Distributed**

Cosuppression in Drosophila: Gene Silencing of *Alcohol dehydrogenase* by *white-Adh* Transgenes Is *Polycomb* Dependent

JOURNAL OF AGRICULTURAL RESEARCH

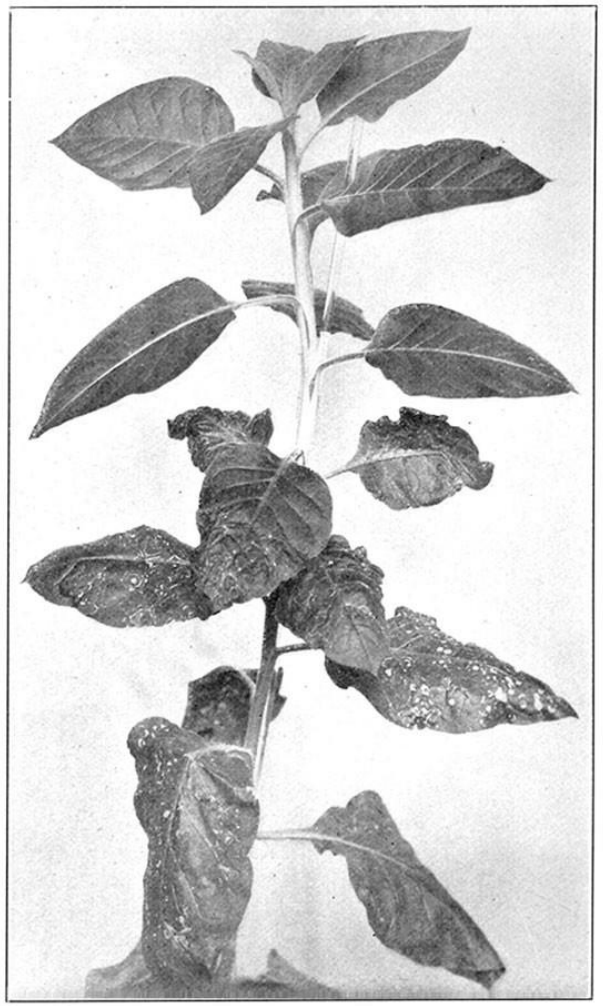
VOL. 37 WASHINGTON, D. C., AUGUST 1, 1928 No. 3

HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

By S. A. WINGARD[2]

Associate Plant Pathologist, Virginia Agricultural Experiment Station

INTRODUCTION



insight review articles

RNA silencing in plants

David Baulcombe

The Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK (e-mail: david.baulcombe@sainsbury-laboratory.ac.uk)

There are at least three RNA silencing pathways for silencing specific genes in plants. In these pathways, silencing signals can be amplified and transmitted between cells, and may even be self-regulated by feedback mechanisms. Diverse biological roles of these pathways have been established, including defence against viruses, regulation of gene expression and the condensation of chromatin into heterochromatin. We are now in a good position to investigate the full extent of this functional diversity in genetic and epigenetic mechanisms of genome control.

Although RNA silencing has only emerged as a topic of general interest in the past six years, the first RNA silencing paper may have been published as long ago as 1928. In that paper Wingard described tobacco plants in which only the initially infected leaves were necrotic and diseased owing to tobacco ringspot virus[1] (Fig. 1). The upper leaves had somehow become immune to the virus and consequently were asymptomatic and resistant to secondary infection. At the time this 'recovery' was a mystery: there was no obvious way to explain the specificity of the resistance to secondary infection.

Baulcombe 2004. Nature 431, 356–363

Histórico

- **1997:** defesa contra vírus em plantas por inserção de sequencia viral (proteína da capa proteína ou outro gene)
- **Similar a silenciamento gênico**

A Similarity Between Viral Defense and Gene Silencing in Plants SCIENCE

Frank Ratcliff, Bryan D. Harrison, David C. Baulcombe*

Gene silencing in plants, in which an endogenous gene is suppressed by introduction of a related transgene, has been used for crop improvement. Observations that viruses are potentially both initiators and targets of gene silencing suggested that this phenomenon may be related to natural defense against viruses. Supporting this idea, it was found that nepovirus infection of nontransgenic plants induces a resistance mechanism that is similar to transgene-induced gene silencing.

Suppression of Virus Accumulation in Transgenic Plants Exhibiting Silencing of Nuclear Genes

James J. English, Elisabeth Mueller, and David C. Baulcombe[1]

The Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich, NR4 7UH, United Kingdom

The Plant Cell, Vol. 8, 179–188, February 1996

Histórico

- **1998:** injeção de dsRNA silencia gene de músculo *unc-22* em *C. elegans*
- dsRNA >>> RNA fita senso ou antisenso
- Sistêmico e sinal amplificado

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

transcripts. RNA interference has been used in the nematode *Caenorhabditis elegans* to manipulate gene expression[3,4]. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous mRNA and suggesting that there could be a catalytic or amplification component in the interference process.

Craig Mello Andrew Fire

Prêmio Nobel de Medicina / Fisiologia 2006

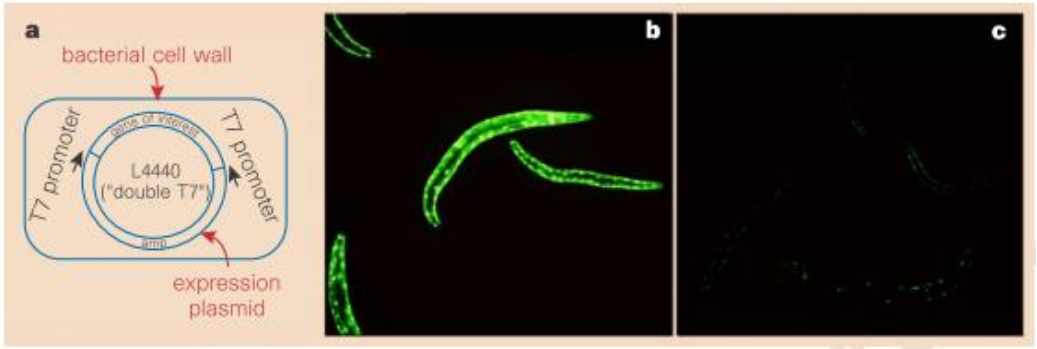
Histórico

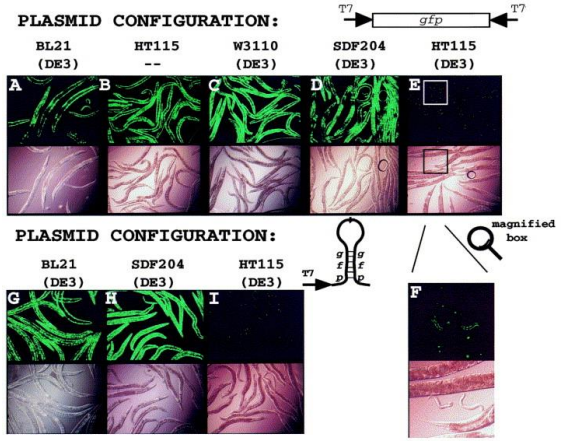
- **1998: ingestão de *E. coli* expressando dsRNA por *C. elegans* leva a RNAi**

Timmons & Fire 1998

Specific interference by ingested dsRNA

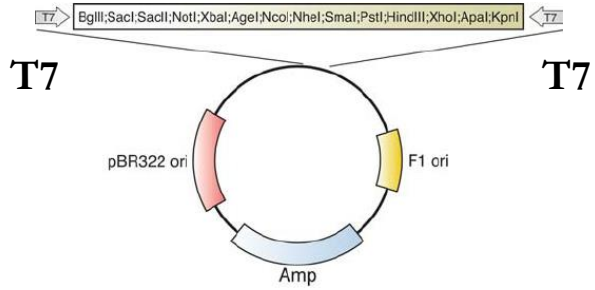
A genetic interference phenomenon in the nematode *Caenorhabditis elegans* has been described in which expression of an individual gene can be specifically reduced by microinjecting a corresponding fragment of double-stranded (ds) RNA[1]. One striking feature of this process is a spreading effect: interference in a broad region of the animal is observed following the injection of dsRNA into the extracellular body cavity. Here we show that *C. elegans* can respond in a gene-specific manner to dsRNA encountered in the environment. *C. elegans* normally feed on bacteria, ingesting and grinding them in the pharynx and subsequently absorbing bacterial contents in the gut. We find that *Escherichia coli* bacteria expressing dsRNAs can confer specific interference effects on the nematode larvae that feed on them.





Timmons et al. 2001 Gene.

We then assessed the ability of dsRNA to interfere with a transgene target. When animals expressing a green fluorescent protein (GFP) transgene were fed bacteria expressing dsRNA corresponding to the *gfp* reporter[1,6], a decrease in GFP fluorescence was observed in about 12% of the population (Fig. 1).



Silenciamento Gênico em Plantas

Silenciamento gênico em plantas derivou evolutivamente:

- Defesa contra infecção por vírus (RNA ou DNA)

- Controle de expressão gênica

- Proteção do genoma de transposons - cromatina

RNAi em insetos

2007: Demonstração de controle por transgenia - Ingestão

Control of coleopteran insect pests through RNA interference

James A Baum[1], Thierry Bogaert[2], William Clinton[1], Gregory R Heck[1], Pascale Feldmann[2], Oliver Ilagan[1], Scott Johnson[1], Geert Plaetinck[2], Tichafa Munyikwa[1], Michael Pleau[1], Ty Vaughn[1] & James Roberts[1,3]

Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol

Ying-Bo Mao[1,2], Wen-Juan Cai[1,2], Jia-Wei Wang[1,2], Gao-Jie Hong[1,2], Xiao-Yuan Tao[1,2], Ling-Jian Wang[1], Yong-Ping Huang[1] & Xiao-Ya Chen[1]

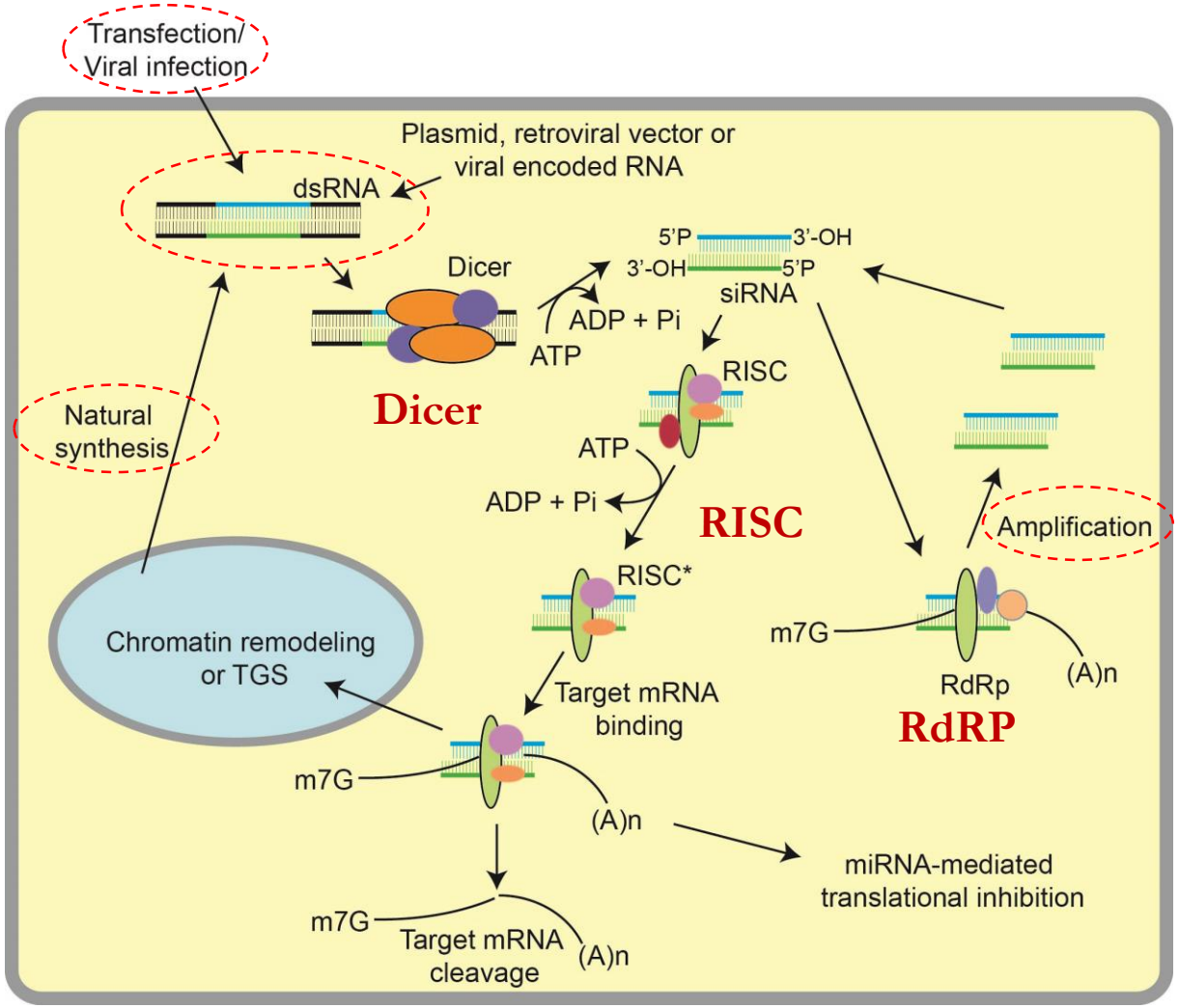
VOLUME 25 NUMBER 11 NOVEMBER 2007

Mecanismo de RNAi

1. Introdução de dsRNA (RNA dupla-fita longo)
2. Digestão por enzima *Dicer (*RNAse III*)*
3. Fragmentos ~19-25 nt - siRNA

   'fita guia' (antissenso) e 'passageira' (senso) -> degradada
4. ***RNA-induced Silencing Complex*** = **RISC**
5. 'RISC-fita guia' identifica mRNA alvo
6. Degradação do mRNA – AGO slicer
7. Amplificação **– RdRP (*RNA-dependent RNA Polymerase*)**

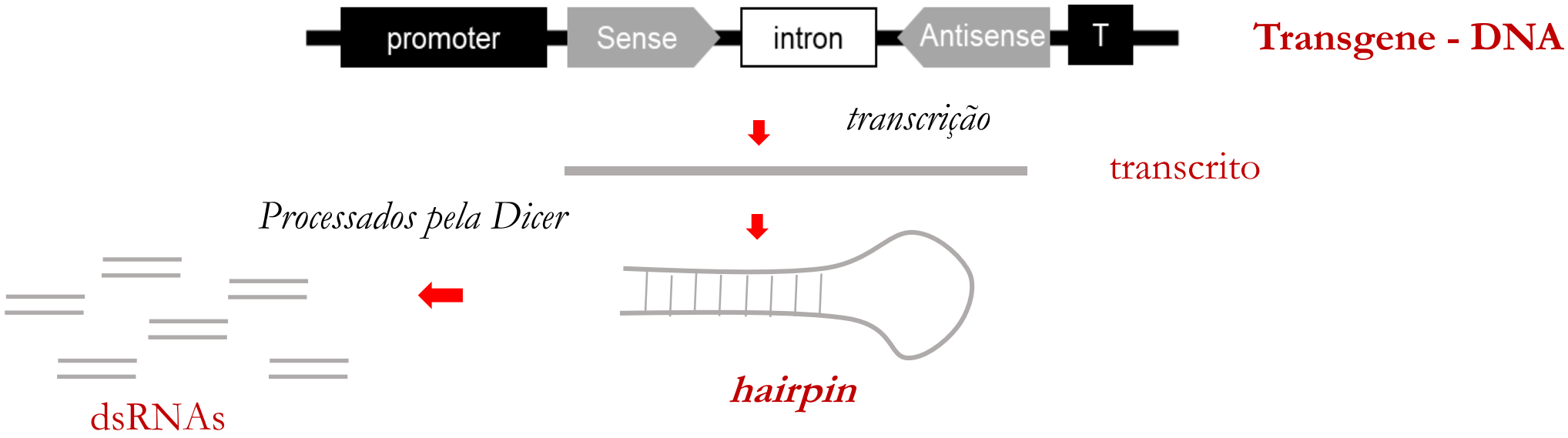
Mecanismo de RNAi



Singh et al. 2015. DOI: 10.5772/61760

Aplicações de RNAi

- **HIGS**: *Host-Induced Gene Silencing*
- Plantas são transformadas com construções que são transcritas em grampo (hairpin) e processados pela maquinaria da planta ou do organismo alvo como siRNA



RNAi em insetos

Produtos comerciais

OPEN ACCESS Freely available online

PLOS ONE

Characterizing the Mechanism of Action of Double-Stranded RNA Activity against Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)

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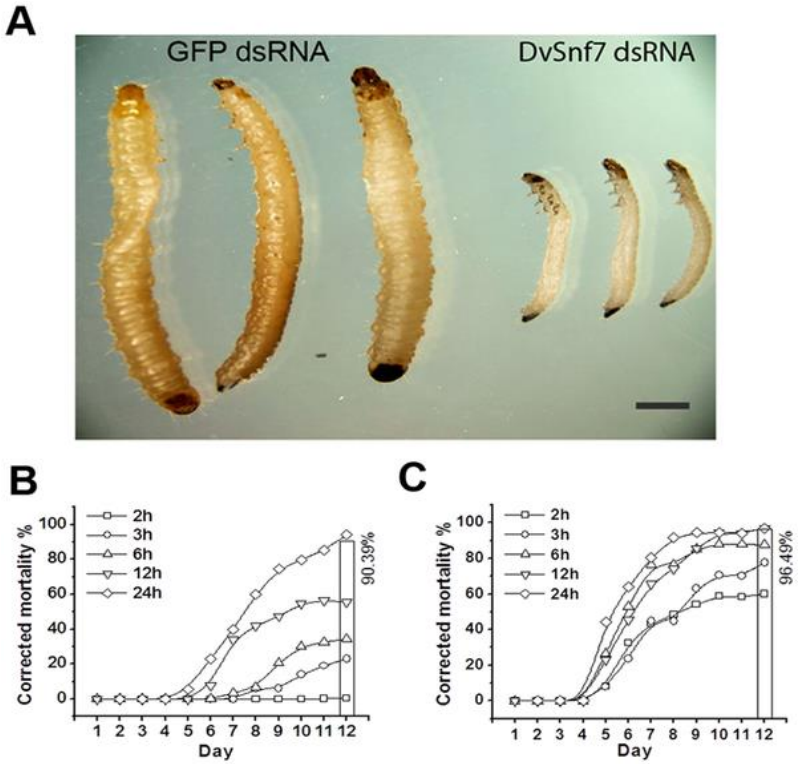
Abstract

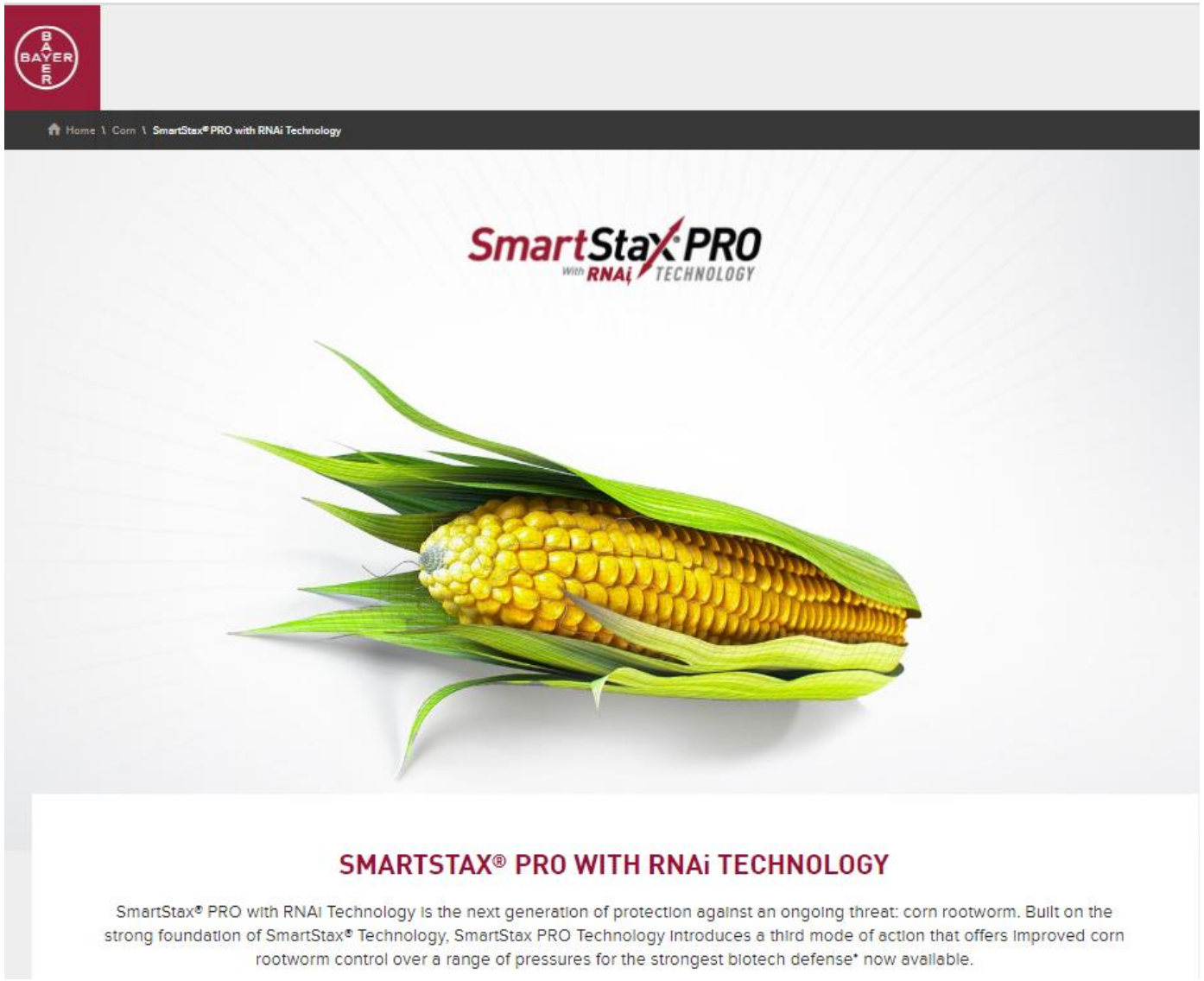
RNA interference (RNAi) has previously been shown to be effective in western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) larvae via oral delivery of synthetic double-stranded RNA (dsRNA) in an artificial diet bioassay, as well as by ingestion of transgenic corn plant tissues engineered to express dsRNA. Although the RNAi machinery components appear to be conserved in Coleopteran insects, the key steps in this process have not been reported for WCR. Here we characterized the sequence of events that result in mortality after ingestion of a dsRNA designed against WCR larvae. We selected the Snf7 ortholog (DvSnf7) as the target mRNA, which encodes an essential protein involved in intracellular trafficking. Our results showed that dsRNAs greater than or equal to approximately 60 base-pairs (bp) are required for biological activity in artificial diet bioassays. Additionally, 240 bp dsRNAs containing a single 21 bp match to the target sequence were also efficacious, whereas 21 bp short interfering (si) RNAs matching the target sequence were not. This result was further investigated in WCR midgut tissues: uptake of 240 bp dsRNA was evident in WCR midgut cells while a 21 bp siRNA was not, supporting the size-activity relationship established in diet bioassays. DvSnf7 suppression was observed in a time-dependent manner with suppression at the mRNA level preceding suppression at the protein level when a 240 bp dsRNA was fed to WCR larvae. DvSnf7 suppression was shown to spread to tissues beyond the midgut within 24 h after dsRNA ingestion. These events (dsRNA uptake, target mRNA and protein suppression, systemic spreading, growth inhibition and eventual mortality) comprise the overall mechanism of action by which DvSnf7 dsRNA affects WCR via oral delivery and provides insights as to how targeted dsRNAs in general are active against insects.



Toxicidade de dsRNA DvSnf7 a larva de Diabrotica virgifera virgifera)

SNF7 – Sucrose Non-Fermenting
Membro do complexo ESCRT-III
endosomal sorting complex required for transport

RNAi em insetos



dvsnf7 – dsRNA – 240 pb
Diabrotica virgifera virgifera

Regulatory Approvals: Country, Year and Type of Approval

Country	Food direct use or processing	Feed direct use or processing	Cultivation domestic or non-domestic use
Argentina	2018		2018
Australia	2015		
Brazil	2016	2016	2016
Canada	2015	2015	2015
Colombia	2016	2016	
Japan	2016	2016	2016 *
Mexico	2015	2015	
New Zealand	2015		
Philippines	2018	2018	
South Korea	2016	2016	
Taiwan	2015		
United States	2014	2014	2015

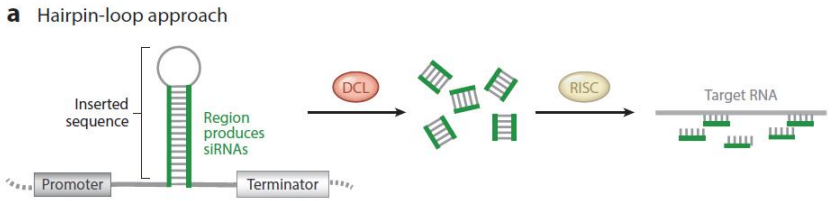
* point mouse arrow over year for notes

Last updated: May 23, 2019

https://www.rnai-technology.com/

Aplicações de RNAi

- Limitações de **HIGS**:
  - Limitações de obter transgenia; nível de expressão; processamento pelo hospedeiro; regulamentação; ...



- Uso de aplicação direta de dsRNA – *Spray-Induced Gene Silencing* (**SIGS**)
- Aplicação de dsRNA por meio de microorganismos
  - Expressão em *E. coli*, levedura, endofíticos
  - Biopesticidas

First Sprayable Double-Stranded RNA-Based Biopesticide Product Targets *Proteasome* Subunit Beta Type-5 in Colorado Potato Beetle (*Leptinotarsa decemlineata*)

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Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) is a major pest of potato and other solanaceous vegetables in the Northern Hemisphere. The insect feeds on leaves and can completely defoliate crops. Because of the repeated use of single insecticide classes without rotating active ingredients, many chemicals are no longer effective in controlling CPB. Ledprona is a sprayable double-stranded RNA biopesticide with a new mode of action that triggers the RNA interference pathway. Laboratory assays with second instar larvae fed Ledprona showed a dose–response where 25×10^{-6} g/L of dsPSMB5 caused 90% mortality after 6 days of initial exposure. We also showed that exposure to Ledprona for 6 h caused larval mortality and decreased target messenger RNA (mRNA) expression. Decrease in PSMB5 protein levels was observed after 48 h of larval exposure to Ledprona. Both PSMB5 mRNA and protein levels did not recover over time. Ledprona efficacy was demonstrated in a whole plant greenhouse trial and performed similarly to spinosad. Ledprona, currently pending registration at EPA, represents a new biopesticide class integrated pest management and insecticide resistance management programs directed against CPB.

Primeiro produto comercial derivado de dsRNA (*proteasome subunit β5*) Ledprona (Calantha)

GreenLight Biosciences

Rodrigues et al., 2021

ESTUDO DIRIGIDO

1 Resolução Normativa 16
2. Técnicas Inovadoras de Melhoramento
3. Componentes de CRISPR/Cas
4. Considerações de Biossegurança de CRISPR
5. Aplicação do CRISPR-Cas9

Leitura recomendada

https://innovativegenomics.org/crisprpedia/