TÉCNICAS INOVADORAS DE MELHORAMENTO DE PRECISÃO

Aula 10

LGN0232 – Genética molecular

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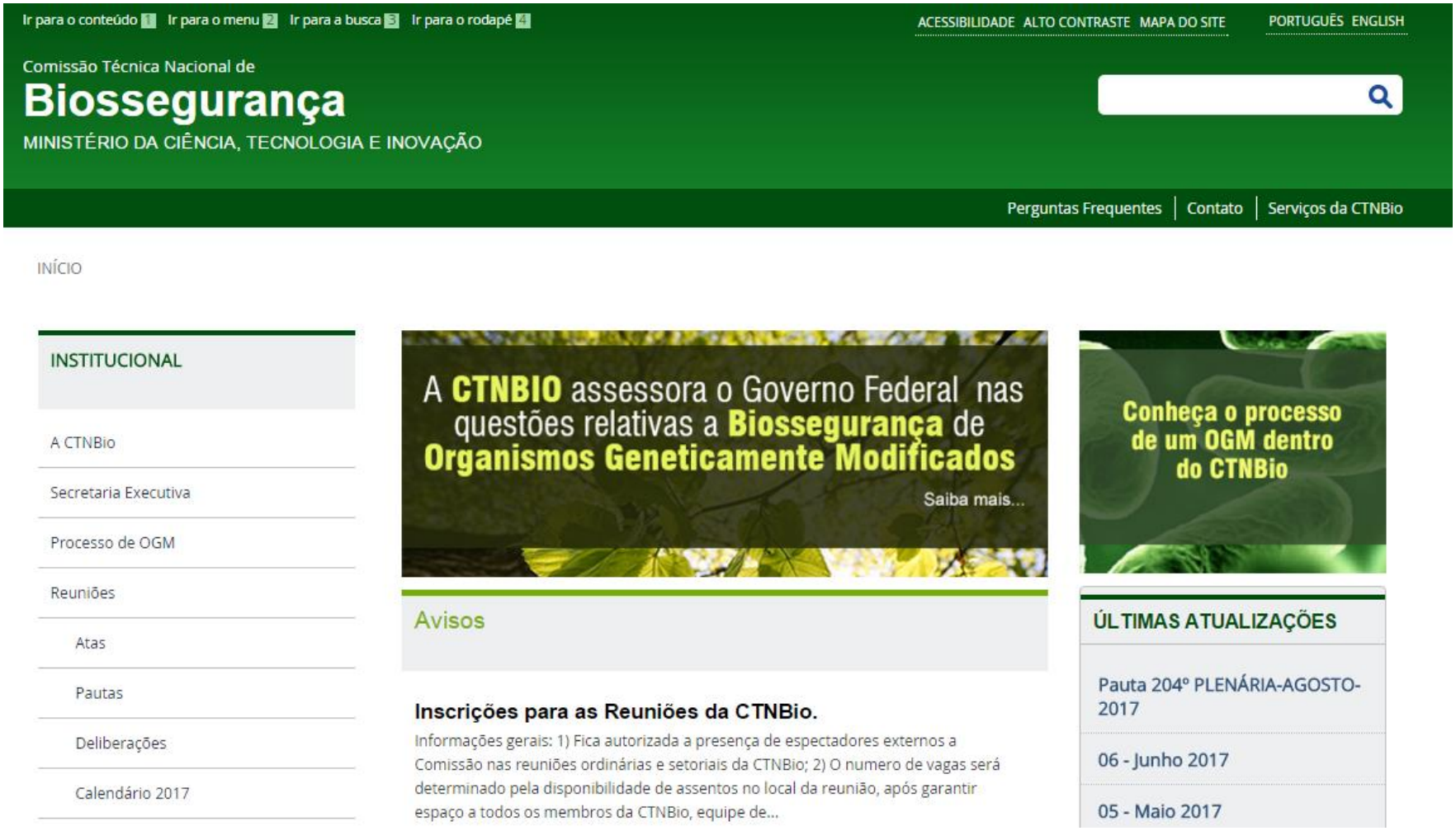
QUEM CONTROLA A LIBERAÇÃO DE ORGANISMOS GENETICAMENTE MODIFICADOS?

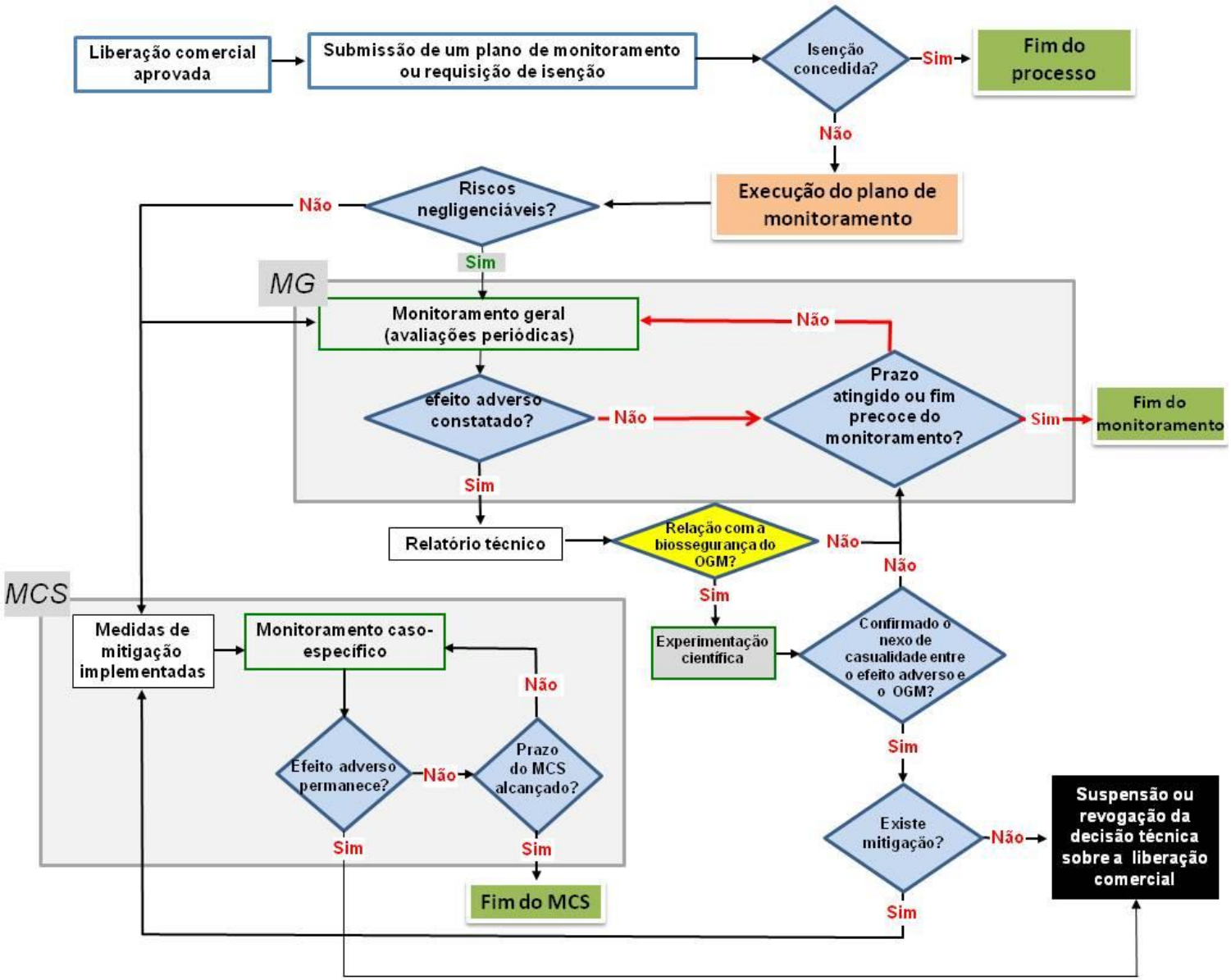
TODO ORGANISMO GENETICAMENTE MODIFICADO É UM TRANSGÊNICO?

ELES SÃO SEGUROS?

TRANSGÊNICOS
Geneticamente Modificados

http://ctnbio.mcti.gov.br/




Liberação comercial aprovada
Submissão de um plano de monitoramento ou requisição de isenção
Isenção concedida?
Sim
Fim do processo
Não
Execução do plano de monitoramento
Riscos negligenciáveis?
Não
Sim
MG
Monitoramento geral (avaliações periódicas)
Não
efeito adverso constatado?
Não
Prazo atingido ou fim precoce do monitoramento?
Sim
Fim do monitoramento
Sim
Relatório técnico
Relação com a biossegurança do OGM?
Não
Não
Sim
MCS
Medidas de mitigação implementadas
Monitoramento caso-específico
Não
Efeito adverso permanece?
Não
Prazo do MCS alcançado?
Sim
Sim
Fim do MCS
Experimentação científica
Confirmado o nexo de casualidade entre o efeito adverso e o OGM?
Sim
Existe mitigação?
Não
Sim
Suspensão ou revogação da decisão técnica sobre a liberação comercial

Liberações Comerciais

Liberações Comerciais

Última atualização 10/06/15 10:51 | 5 Subpastas | 0 Documentos

▼ Subpastas

Nome ▼
Vacinas Subpastas: Parecer Técnico nº 099-2004, Parecer Técnico nº 1300-2008, Parecer Técnico nº 1427-2008, Parecer Técnico nº 1591-2008, Parecer Técnico nº 2146-2009, Mais »
Plantas Plantas Subpastas: Algodão, Eucalipto, Feijão, Milho, Soja, Mais »
Outros Subpastas: Parecer Técnico nº 261-470_2004 - Importação e Liberação Comercial de Enzimas - Processo 01200.00374, Parecer Técnico nº 3964 - 2014 - OX513A de Aedes aegypti
Microorganismos Subpastas: Parecer Técnico nº 2281 - 2010, Parecer Técnico nº 3287 - 2012, Parecer Técnico nº 3775 - 2013, Parecer Técnico nº 3877 - 2013, Parecer Técnico nº 4203 - 2014, Mais »
English Version Subpastas: Crops, Microorganisms, Others, Vacccines

Mostrando 5 resultados.

Nome ▼

Tabela de Plantas - Uso Comercial
Plantas Geneticamente modificadas aprovadas para Comercialização

Soja
Subpastas: Comunicado nº 54, Parecer Técnico nº 2236-2009, Parecer Técnico nº 2273-2010, Parecer Técnico nº 2286-2010, Parecer Técnico nº 2542-2010, Mais »

Milho
Subpastas: Parecer Técnico nº 0987 - 2007, Parecer Técnico nº 1100 - 2007, Parecer Técnico nº 1255 - 2008, Parecer Técnico nº 1596 - 2008, Parecer Técnico nº 1597 - 2008, Mais »

Feijão
Subpastas: Parecer Técnico nº 3024-2011

Eucalipto
Subpastas: Parecer Técnico nº 4408-2015

Algodão
Algodão
Subpastas: Parecer Técnico nº 0513-2005, Parecer Técnico nº 1521 - 2008, Parecer Técnico nº 1598 - 2008, Parecer Técnico nº 1757 - 2009, Parecer Técnico nº 1832-2009, Mais »

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...

Ler mais »

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 »

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.

TRECHOS IMPORTANTES

- 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;

....

- ...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....

RNAi??

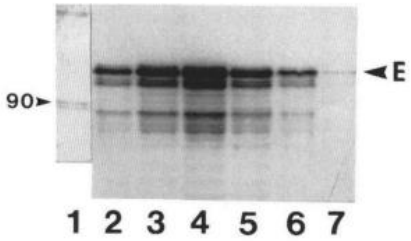
Como funciona?

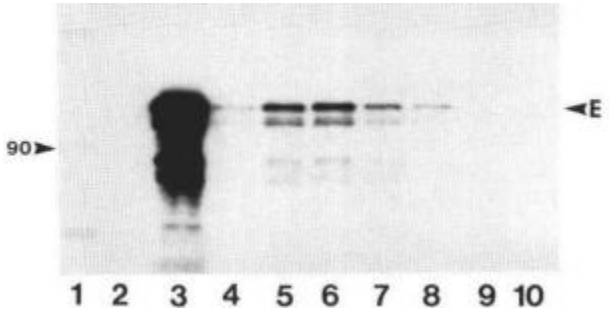
O início de tudo… 1990s

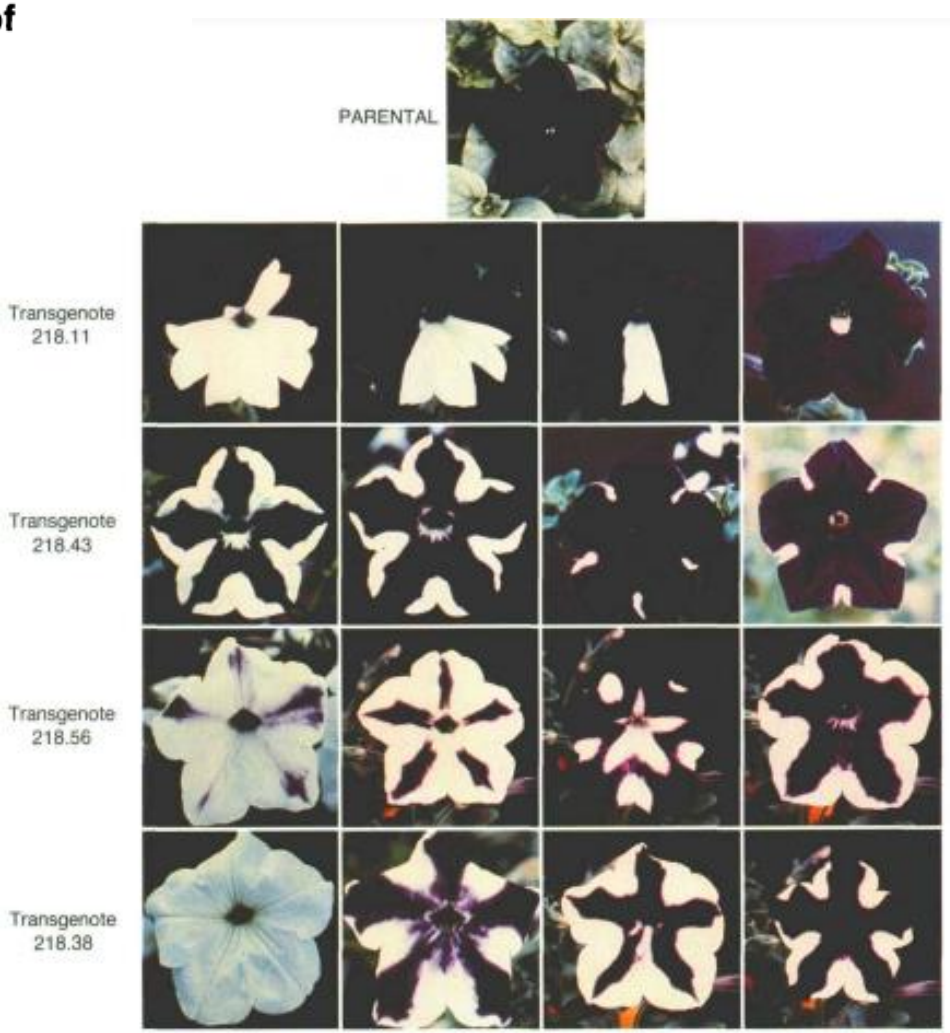
The Plant Cell, Vol. 2, 279–289, April 1990 © 1990 American Society of Plant Physiologists

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







doi: 10.1105/tpc.2.4.279

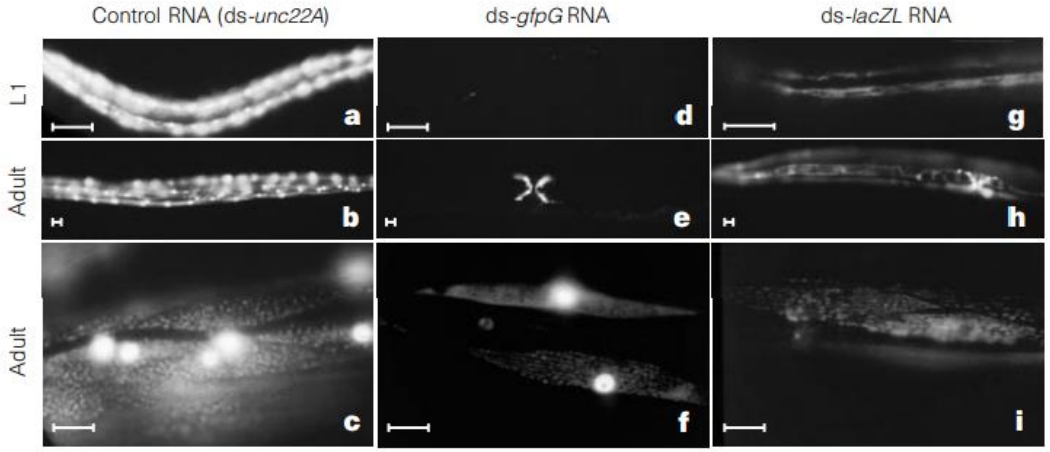
1998

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‡

** Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA*
† Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA
‡ Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA

- Injeção de dsRNA silencia gene de músculo unc-22 em *C. elegans.*
- dsRNA>>> RNA fita senso ou antisense
- Sistêmico e sinal amplificado



https://doi.org/10.1038/35888

Published: 29 October 1998

Specific interference by ingested dsRNA

Lisa Timmons & Andrew Fire

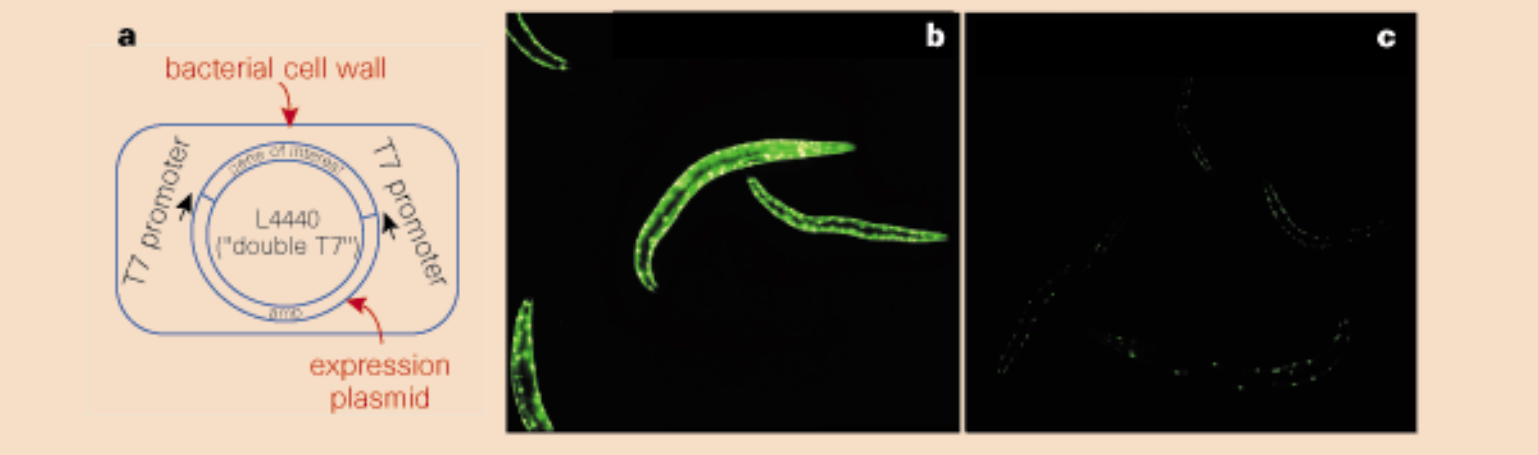
Nature **395**, 854 (1998) | Cite this article

Abstract

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.

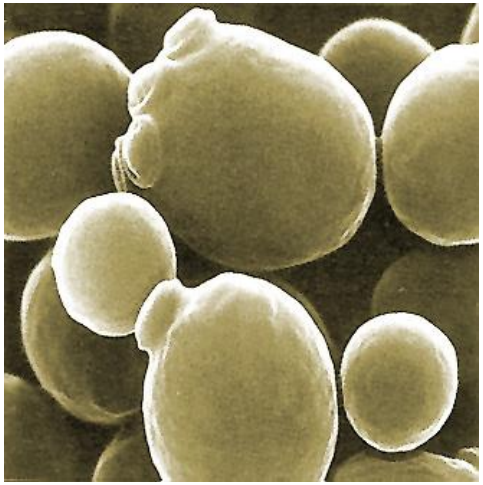
Ingestão de *E.coli* expressando dsRNA por *C. elegans* -> silenciamento

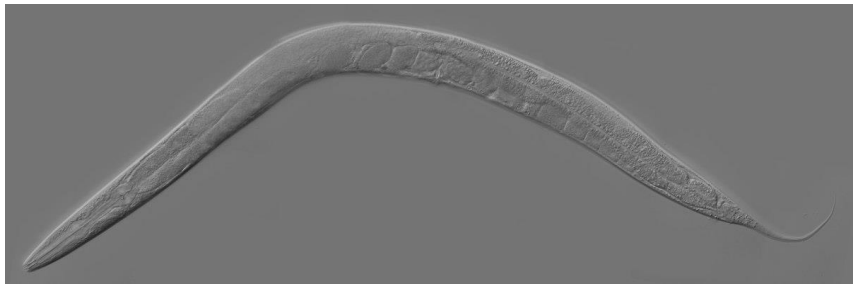
- O gene *fem-1* codifica um componente tardio da via de determinação do sexo *do C. elegans* .
- Mutações nulas ou injeção de dsRNA impedem a produção de espermatozoides - produzem fêmeas estéreis.



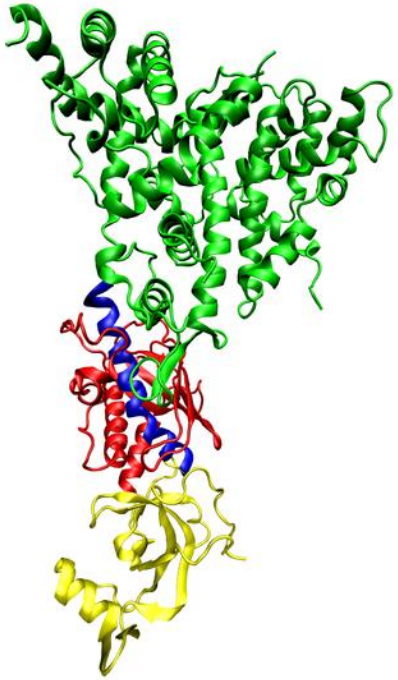
DOI: 10.1038/27579

Sistema presente em diferentes organismos modelo…

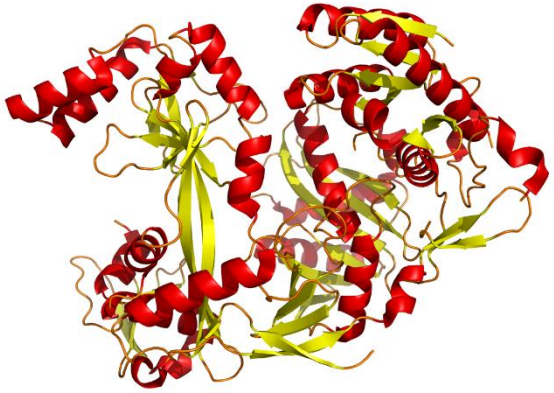


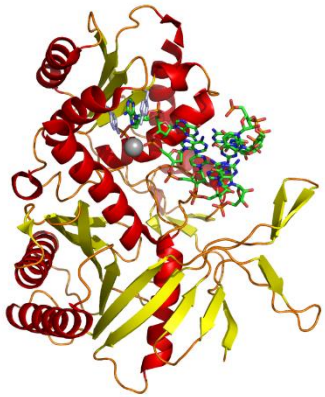


ALGUMAS PROTEÍNAS CHAVES…

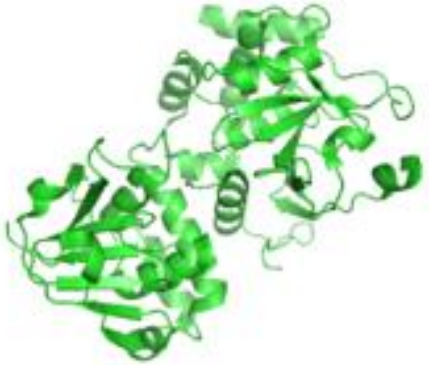


Dicer



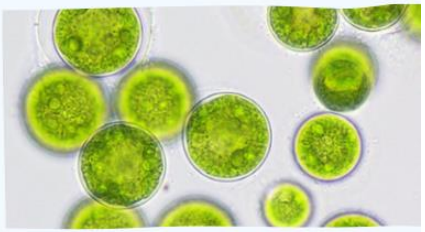


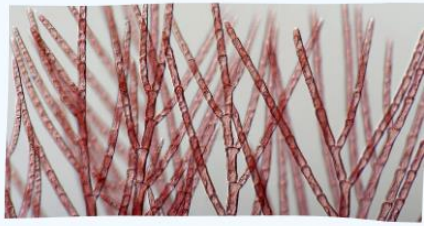
Argonatas

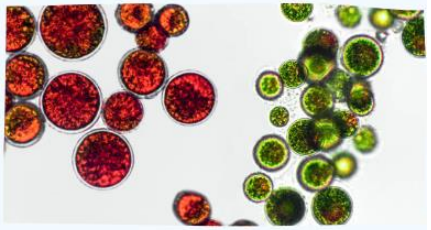


RNA-dependent RNA polymerase (RdRP)

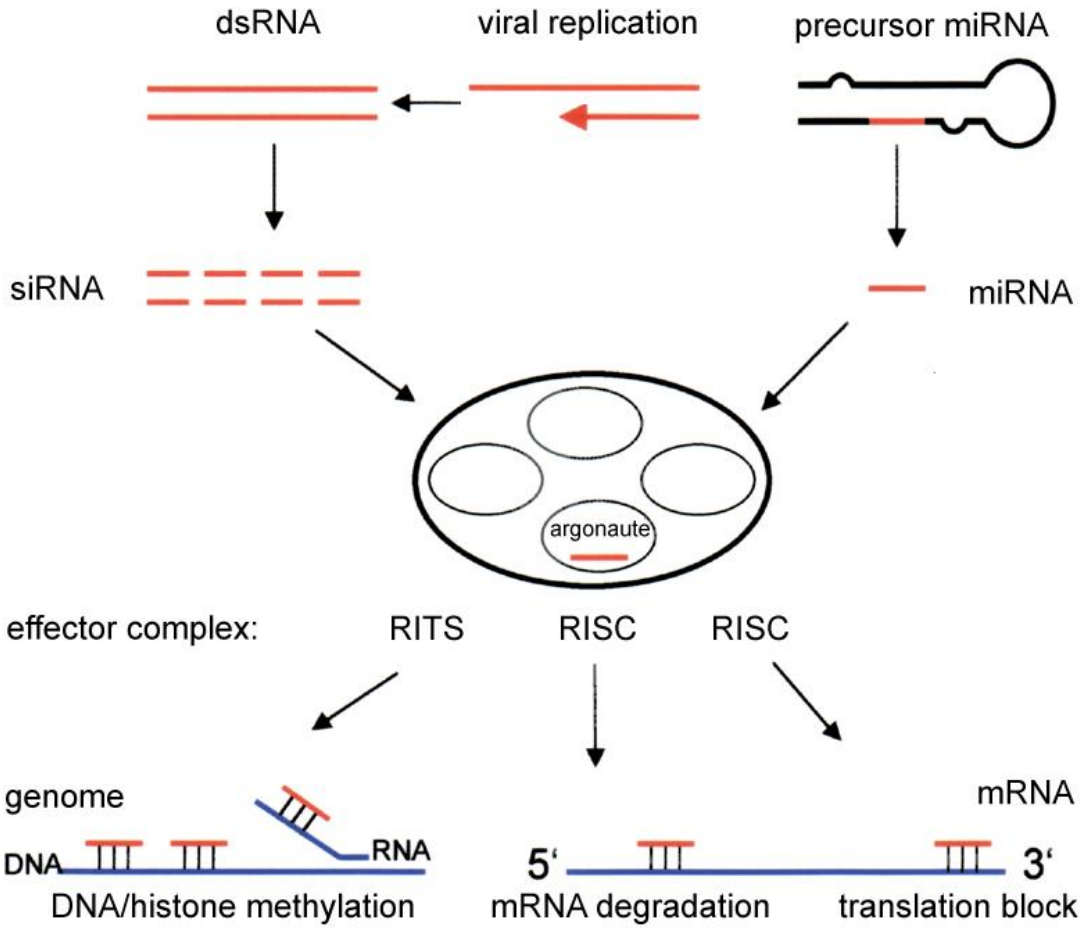
Species	Genome size (Mb)	AGO-Piwi	Dicer	RDR
Glaucophyta				
Cyanophora paradoxa	70.0	0	0	1
Rhodophyta				
Galdieria sulphuraria	13.7	0	0	0
Cyanidioschyzon merolae	16.5	0	0	0
Porphyridium purpureum	19.7	3	1	3
Chlorophyta				
Ostreococcus tauri	12.5	0	0	0
Ostreococcus lucimarinus	13.2	0	0	0
Bathycoccus prasinos	15.1	0	0	0
Micromonas sp. RCC299	20.9	1	0	0
Micromonas pusilla CCMP1545	21.9	0	0	0
Chlorella variabilis NC64A	46.0	1	1	0
Chlorella sorokiniana	56.8	1	1	0
Coccomyxa subellipsoidea	48.8	2	1?	1
Chlamydomonas reinhardtii	120.0	3	3	0
Volvox carteri	138.0	2	1	0







doi: 10.1128/EC.05106-11

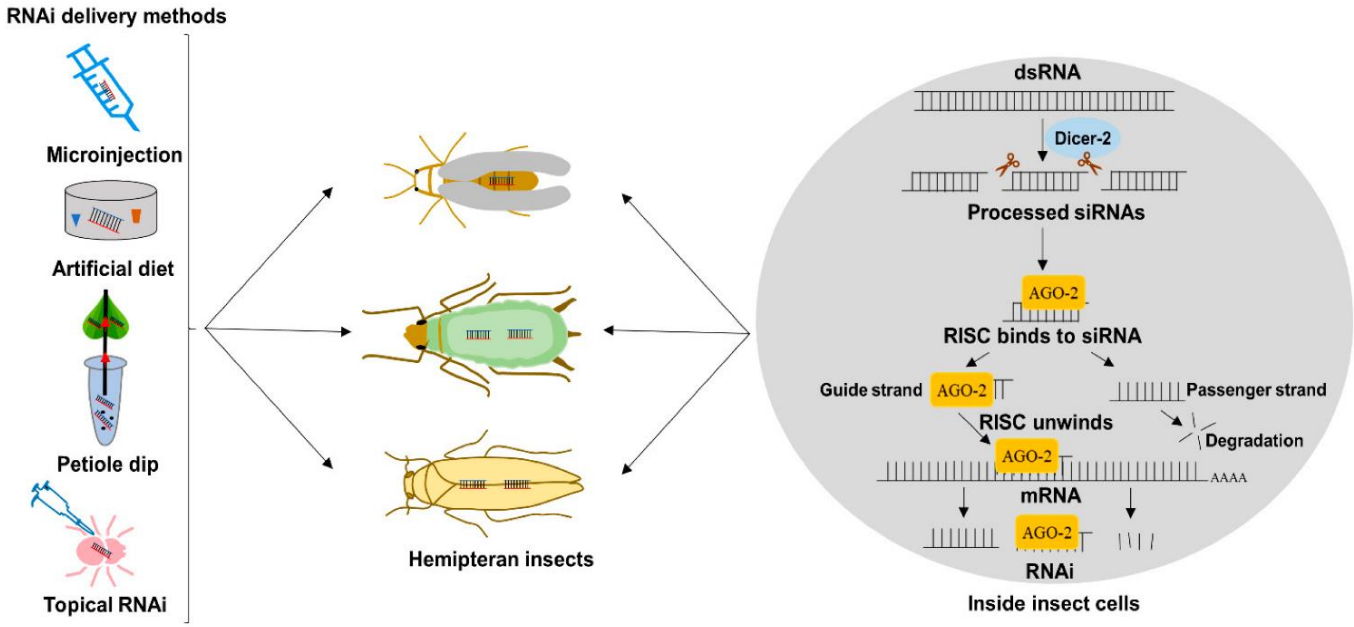


A enzima Dicer reconhece dsRNA para formar siRNA ou miRNA. Esses RNAs são incorporados no RNA-induced silencing complex (RISC), que se liga ao mRNA.

RNAi em insetos

Formas de entrega:

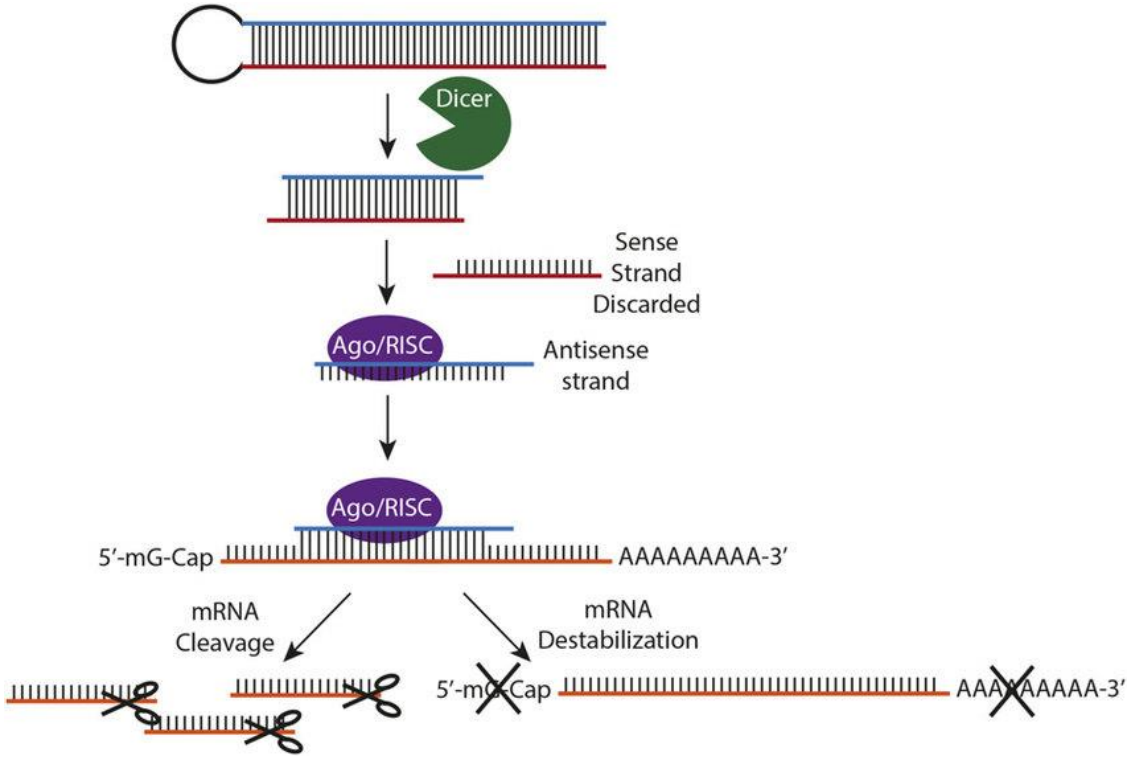
- Microinjeção
- Entrega com NPs
- Soaking
- Ingestão – exemplo *E.coli e C. elegans*.



https://doi.org/10.3390/insects11090557

- HIGs (Host-Induced Gene Silencing) – Plantas transformadas com construções que são transcritas em hairpin – processados pela maquinaria da planta ou inseto ao se alimentar.

- SIGs (Spray-Induced gene silencing)



doi: 10.1111/mpp.12866

http://profissaobiotec.com.br/primeira-planta-transgenica-contendo-tecnologia-rnai-com-atividade-inseticida-e-aprovada-nos-eua/

Interfere na regulação da expressão gênica de patógeno

Published: 04 November 2007

Control of coleopteran insect pests through RNA interference

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

Nature Biotechnology **25**, 1322–1326 (2007) | Cite this article

18k Accesses | **1244** Citations | **59** Altmetric | Metrics

Abstract

Commercial biotechnology solutions for controlling lepidopteran and coleopteran insect pests on crops depend on the expression of *Bacillus thuringiensis* insecticidal proteins[1,2], most of which permeabilize the membranes of gut epithelial cells of susceptible insects[3]. However, insect control strategies involving a different mode of action would be valuable for managing the emergence of insect resistance. Toward this end, we demonstrate that ingestion of double-stranded (ds)RNAs supplied in an artificial diet triggers RNA interference in several coleopteran species, most notably the western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte. This may result in larval stunting and mortality. Transgenic corn plants engineered to express WCR dsRNAs show a significant reduction in WCR feeding damage in a growth chamber assay, suggesting that the RNAi pathway can be exploited to control insect pests via *in planta* expression of a dsRNA.

https://doi.org/10.1038/nbt1359

Published: 04 November 2007

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

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

Nature Biotechnology **25**, 1307–1313 (2007) | Cite this article

11k Accesses | **970** Citations | **31** Altmetric | Metrics

Abstract

We identify a cytochrome P450 gene (*CYP6AE14*) from cotton bollworm (*Helicoverpa armigera*), which permits this herbivore to tolerate otherwise inhibitory concentrations of the cotton metabolite, gossypol. *CYP6AE14* is highly expressed in the midgut and its expression correlates with larval growth when gossypol is included in the diet. When larvae are fed plant material expressing double-stranded RNA (dsRNA) specific to *CYP6AE14*, levels of this transcript in the midgut decrease and larval growth is retarded. Both effects are more dramatic in the presence of gossypol. As a glutathione-*S*-transferase gene (*GST1*) is silenced in *GST1* dsRNA–expressing plants, feeding insects plant material expressing dsRNA may be a

https://doi.org/10.1038/nbt1352

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

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

Renata Bolognesi, Parthasarathy Ramaseshadri, Jerry Anderson, Pamela Bachman, William Clinton, Ronald Flannagan, Oliver Ilagan, Christina Lawrence, Steven Levine, William Moar, Geoffrey Mueller, Jianguo Tan, Joshua Uffman, [...], Gerrit Segers

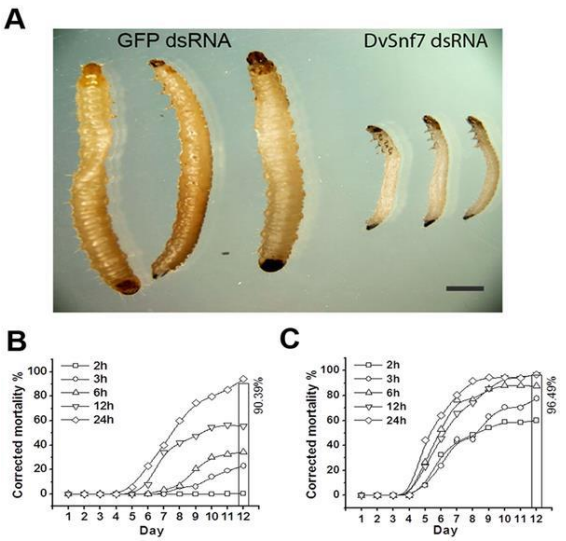
[view all]

Published: October 11, 2012 • https://doi.org/10.1371/journal.pone.0047534

Article | Authors | Metrics | Comments | Media Coverage

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



Snf7 (DvSnf7) como mRNA alvo - codifica uma proteína essencial envolvida no tráfego intracelular.

https://doi.org/10.1371/journal.pone.0047534

PESQUISA IRÁ CRIAR BIODEFENSIVOS DE USO TÓPICO PARA CONTROLE DE PLANTAS DANINHAS, INSETOS-PRAGA E DOENÇAS PARA AS PRINCIPAIS CULTURAS AGRÍCOLAS

https://www.agrointeracao.com.br/noticias/2020/09/23/pesquisa-ira-criar-biodefensivos-de-uso-topico-para-controle-de-plantas-daninhas-insetos-praga-e-doencas-para-as-principais-culturas-agricolas/

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

Thais B. Rodrigues[1], Sambit K. Mishra[1], Krishnakumar Sridharan[1], Ethann R. Barnes[1], Andrei Alyokhin[3], Rich Tuttle[1], Wimalanathan Kokulapalan[2], David Garby[2], Nicholas J. Skizim[2], Yu-wen Tang[2], Brian Manley[1], Lorenzo Aulisa[2], Ronald D. Flannagan[1], Carole Cobb[2] and Kenneth E. Narva[1]*

[1]GreenLight Biosciences, Research Triangle Park, NC, United States, [2]School of Biology and Ecology, University of Maine, Orono, ME, United States, [3]GreenLight Biosciences, Medford, MA, United States

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Citation:

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.

- besouro da batata do Colorado *Leptinotarsa decemlineata*
- Resistente a muitos pesticidas

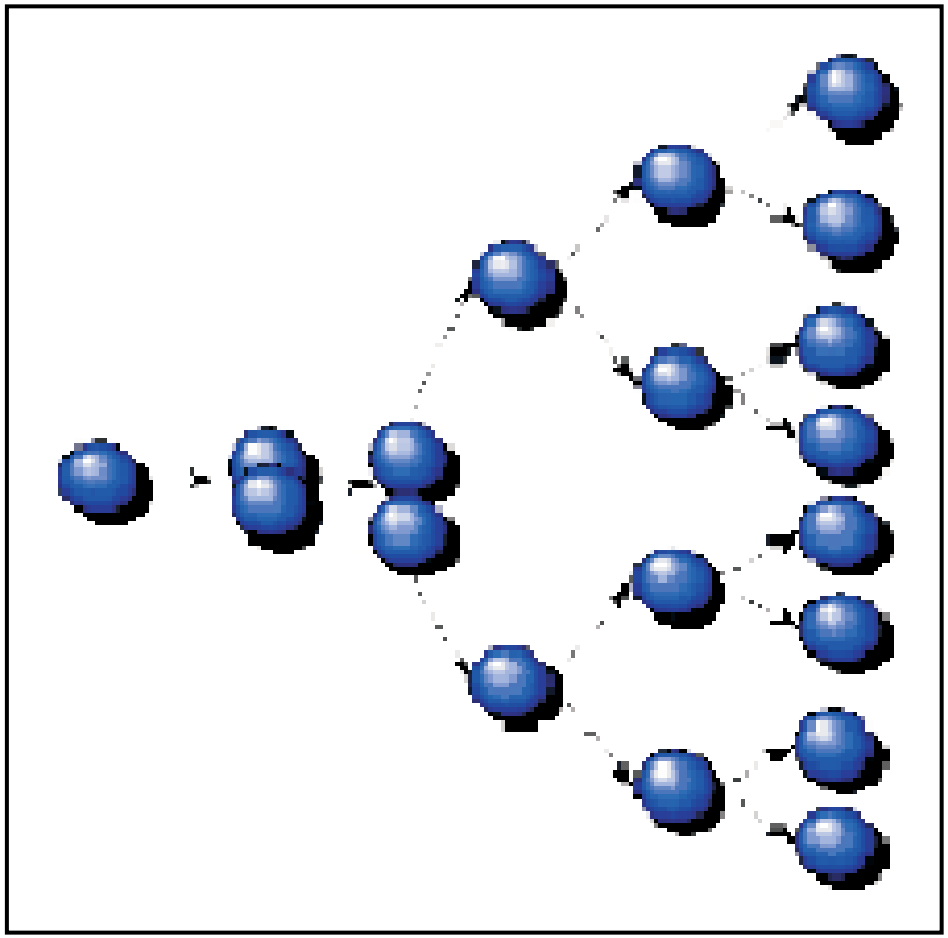
Ledprona é um biopesticida de RNA de fita dupla pulverizável

https://doi.org/10.3389/fpls.2021.728652

CRISPR-Cas??

Edição gênica?

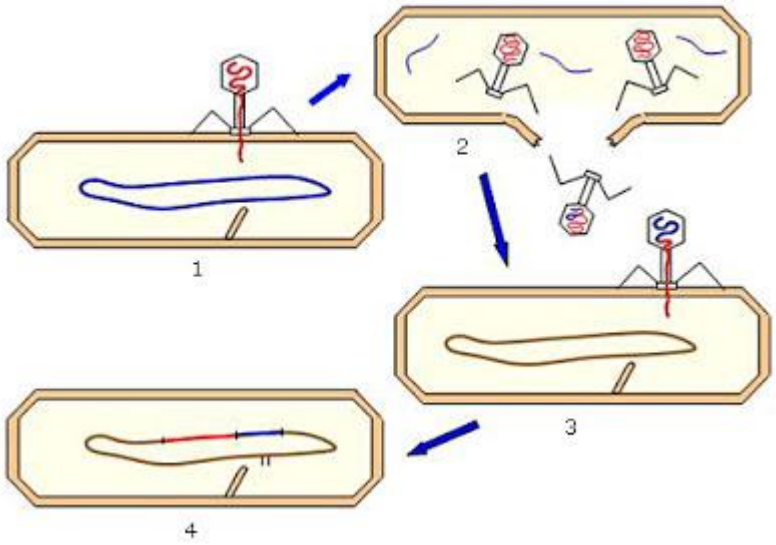
PROCARIOTOS: MODELO CLONAL



Lawrence, 2002.

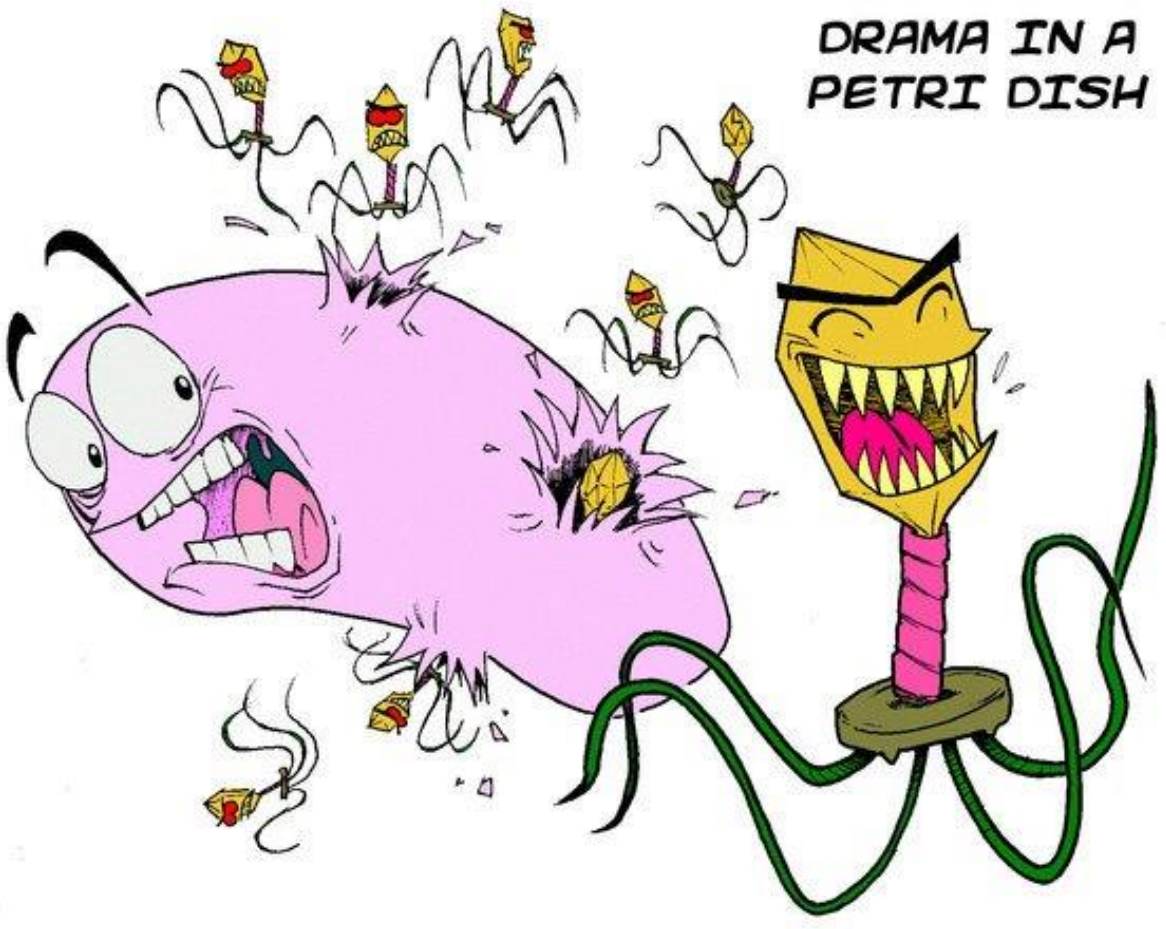
TRANSFERÊNCIA HORIZONTAL DE GENES

Troca de material genético entre células sem parentesco



- **Transformação (DNA livre).**
- **Conjugação (plasmídeos).**
- **Transdução (vírus).**

NA VERDADE É UM FILME DE TERROR...



PROCARIOTOS NÃO SÃO TÃO PASSIVOS

PLOS | PATHOGENS

PEARLS

Battling Phages: How Bacteria Defend against Viral Attack

Kimberley D. Seed*

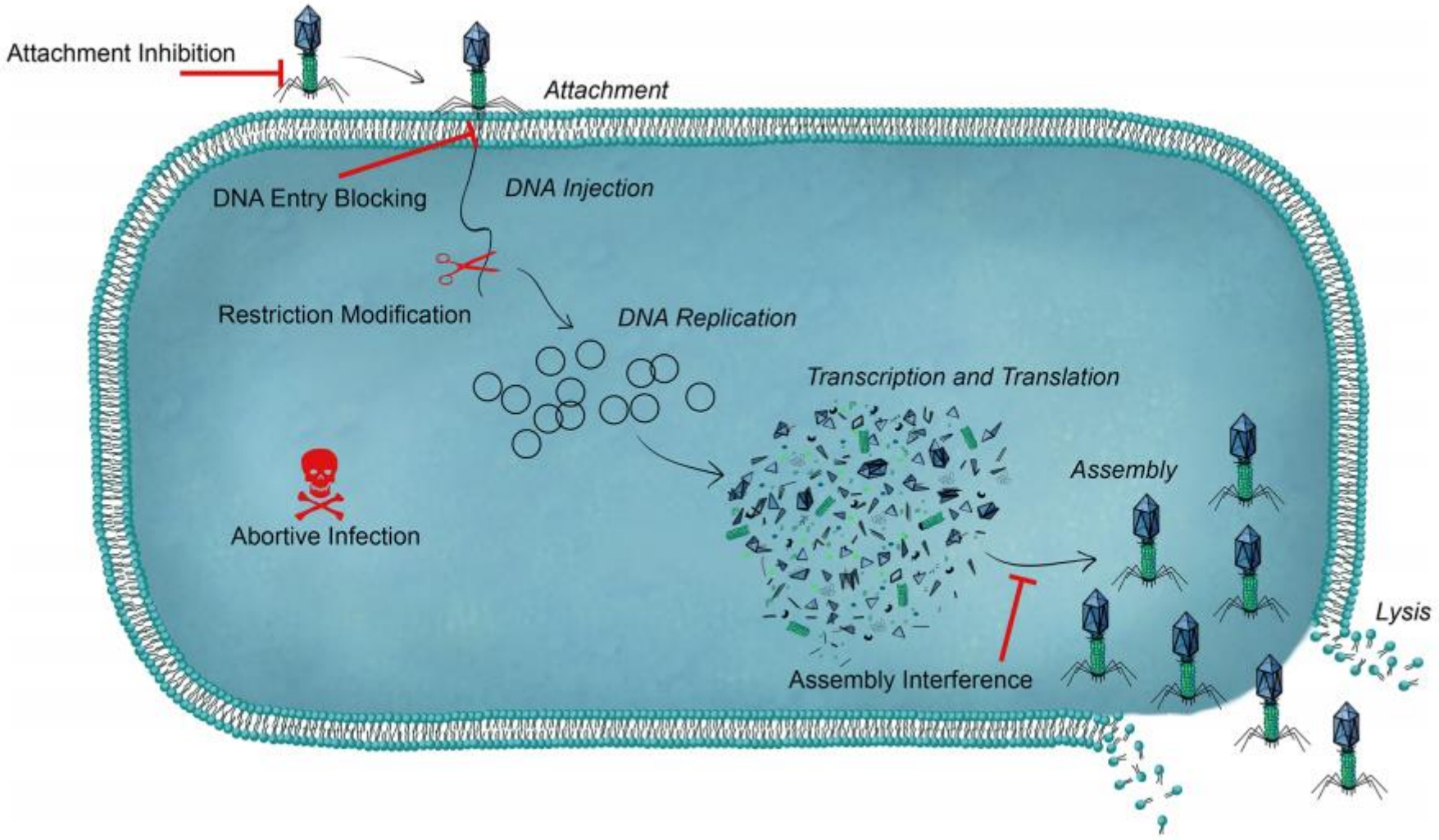
Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan, United States of America

* kdseed@umich.edu

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].

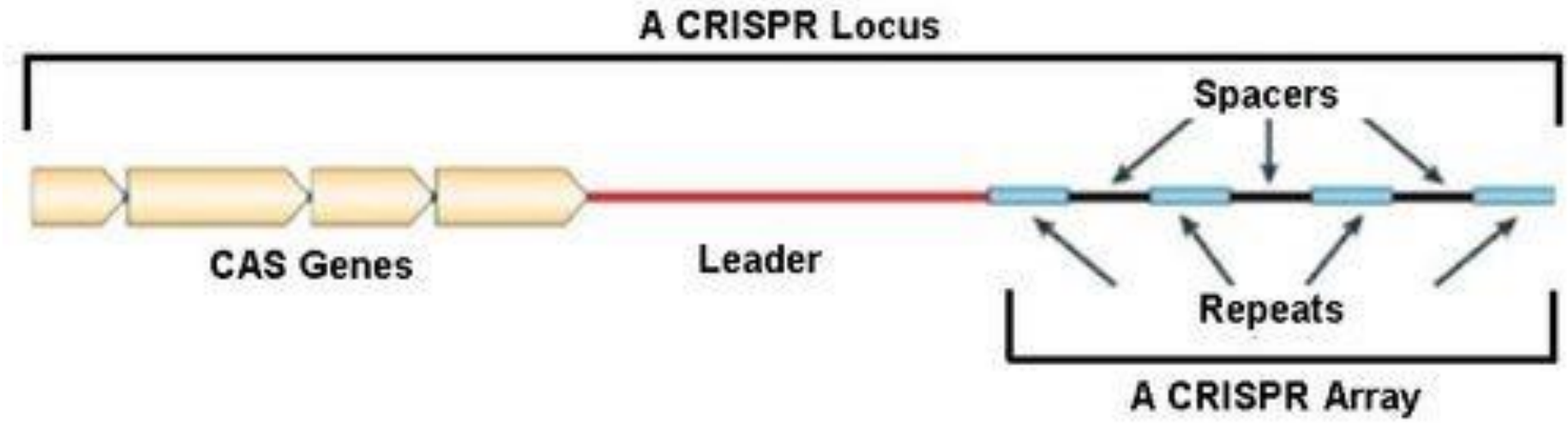
Alguns mecanismos inatos …



E o CRISPR-CAS?

DEFINIÇÃO...

CRISPR–Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) modulos são sistema adapatativos de imunidade presente em muitas arquéias e bactérias.



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

Department of Experimental Chemotherapy, Research Institute for Microbial Diseases, Osaka University, 3-1, Yamadaoka, Suita, Osaka, Japan 565

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"

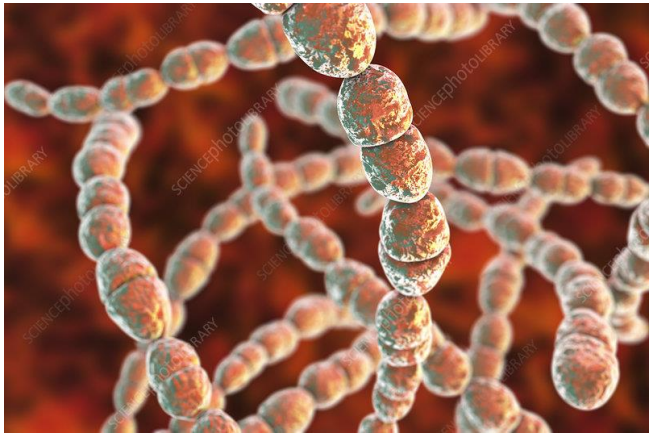
Na realidade ….

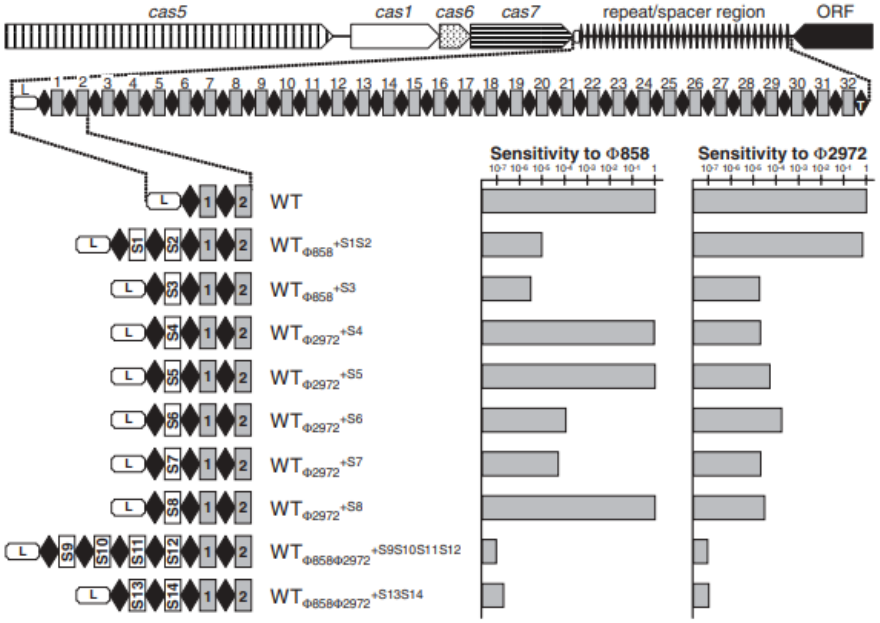
CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

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]*

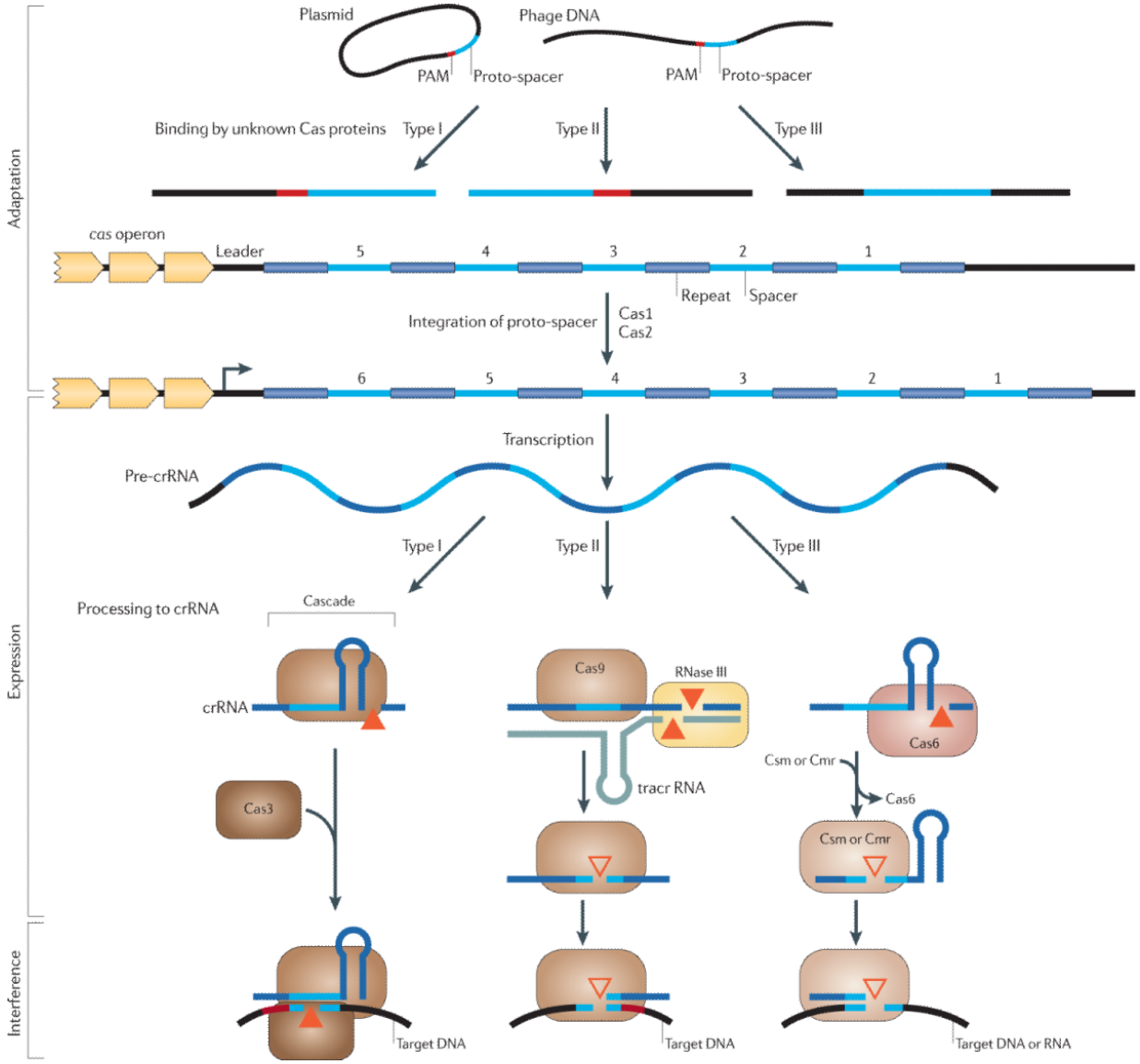
Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences.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

Ensaio com *S. thermophilus*

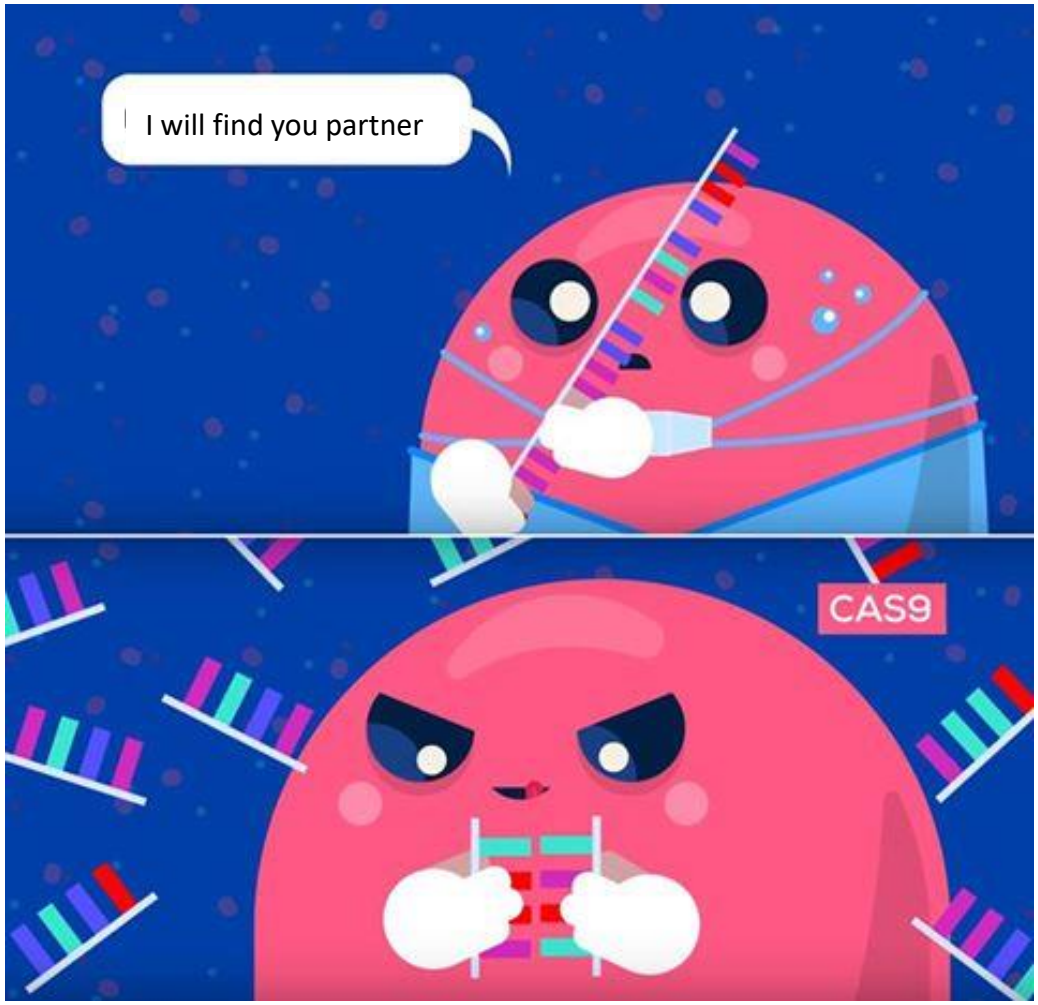




Três estágios da ação do CRISPR–Cas



COMO USAR ISSO EM MEU LABORATÓRIO?



UM DOS PRIMEIROS TRABALHOS...

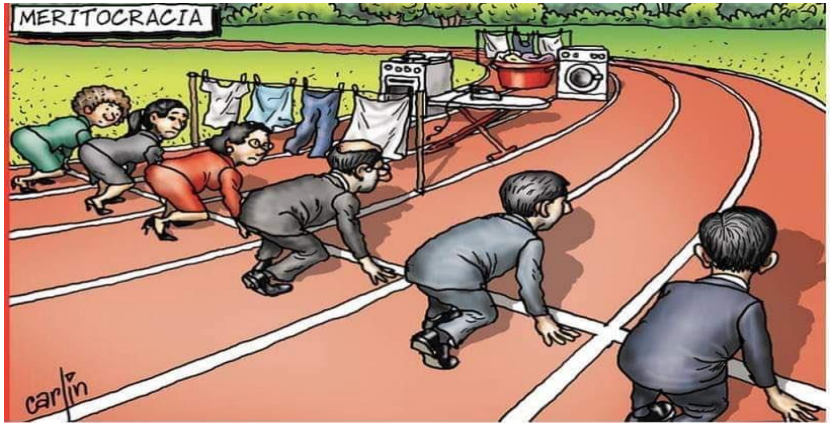
A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

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.

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

GANHADORAS DO PRÊMIO NOBEL DE QUIMICA EM 2020



Quebrando paradigmas….

Published in final edited form as:
Science. 2013 February 15; 339(6121): 823–826. doi:10.1126/science.1232033.

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali[1,5], **Luhan Yang**[1,3,5], **Kevin M. Esvelt**[2], **John Aach**[1], **Marc Guell**[1], **James E. DiCarlo**[4], **Julie E. Norville**[1], and **George M. Church**[1,2,*]

[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA

[2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138, USA

[3]Biological and Biomedical Sciences Program, Harvard Medical School, Boston, MA 02115, USA

[4]Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA

Published in final edited form as:
Science. 2013 February 15; 339(6121): 819–823. doi:10.1126/science.1231143.

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], and **Feng Zhang**[1,†]

BIOTECHNOLOGY

Bitter fight over CRISPR patent heats up

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

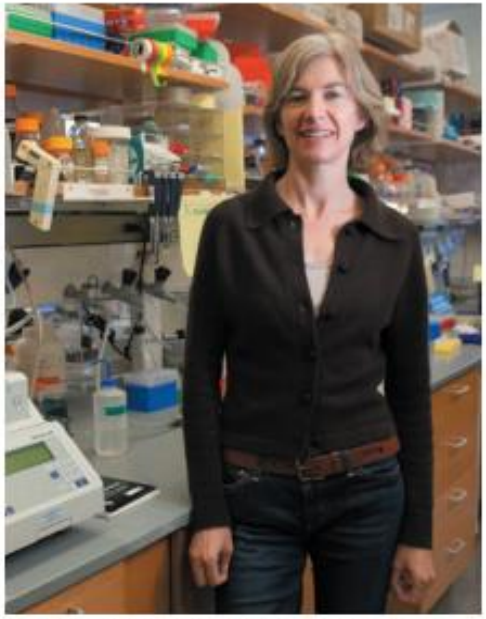
BY HEIDI LEDFORD

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

FEB 21, 2017 @ 05:00 AM 15,210 EDITOR'S PICK

How Much Is a CRISPR Patent License Worth?

Jacob S. Sherkow, CONTRIBUTOR
FULL BIO
Opinions expressed by Forbes Contributors are their own.

Last Wednesday, the Patent Trial and Appeal Board (PTAB) ruled in favor of the Broad Institute in the most monumental biotech patent dispute in decades: a patent "interference" trial over foundational patents covering CRISPR-Cas9, a revolutionary gene-editing technology. A day later—in some truly fortuitous timing—Jorge Contreras of the University of Utah and I published an article in *Science* examining some of the licensing complexities surrounding research institutions' surrogate companies: for-profit biotech companies with exclusive

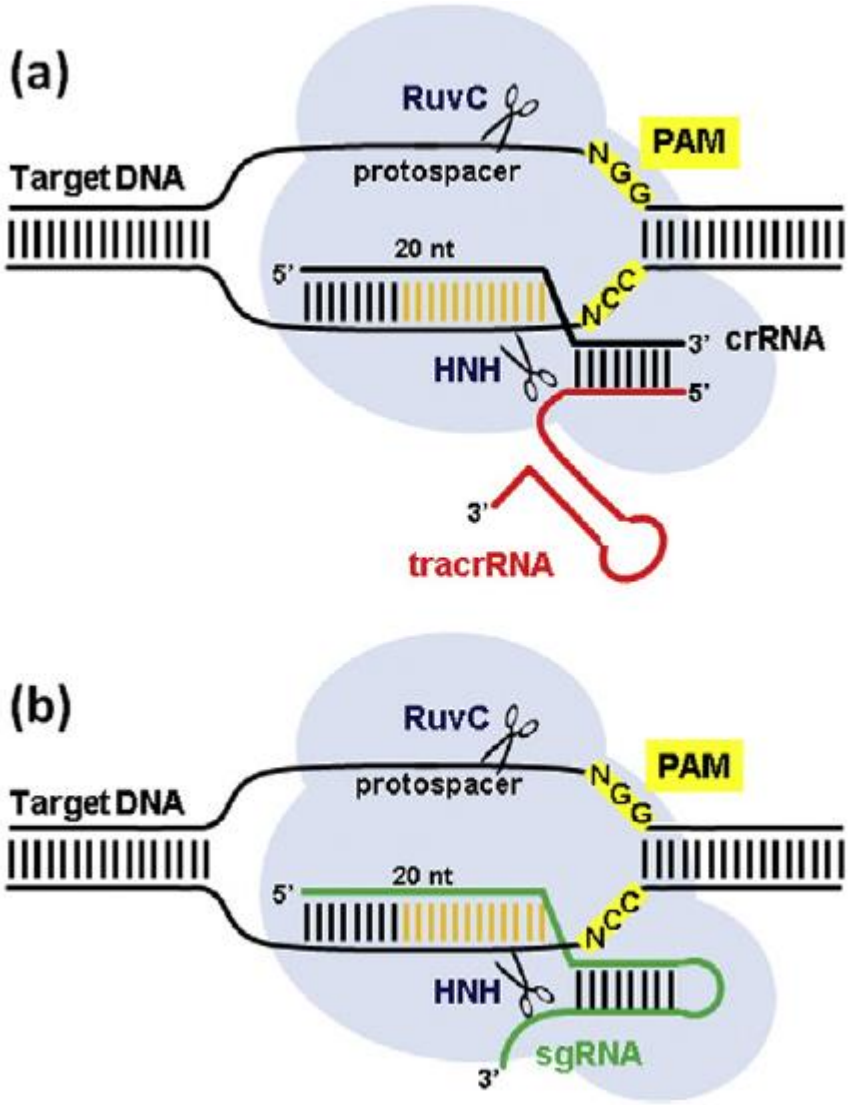
STAT+

CRISPR patent agreement seeks to expand use in crops

By SHARON BEGLEY @sxbegle / OCTOBER 18, 2017

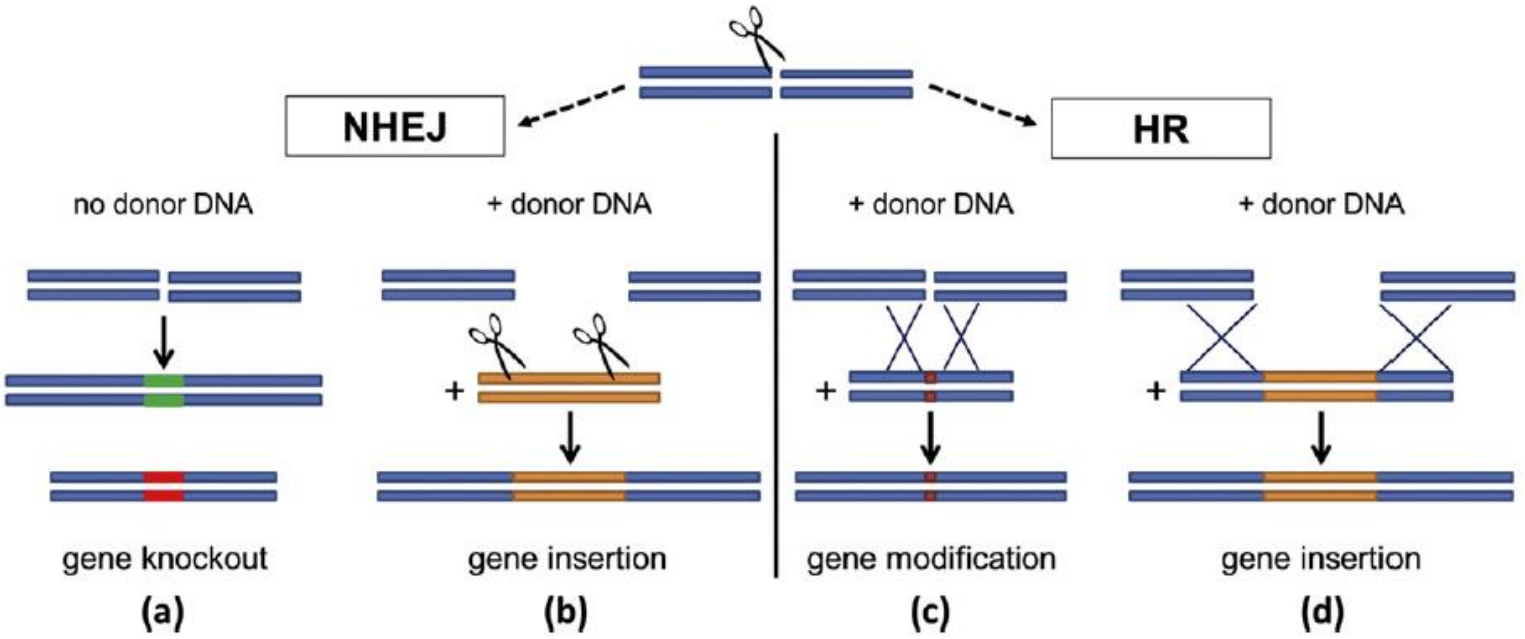
https://www.statnews.com/2017/10/18/crispr-patent-expand-crops/

Otimização do Sistema Crispr-Cas9



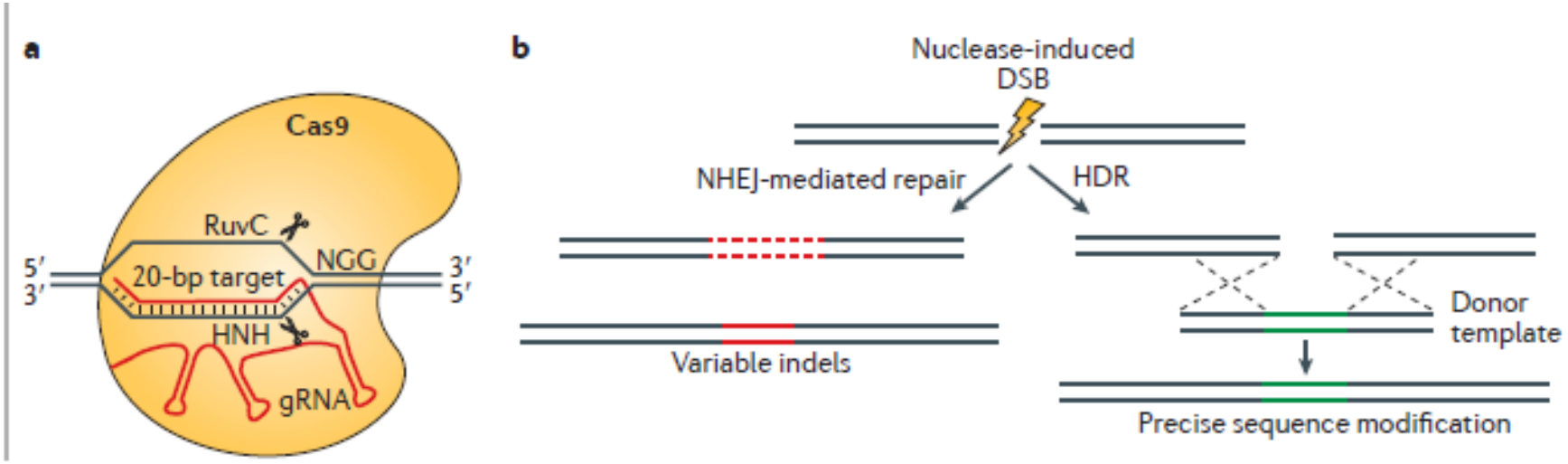
doi: 10.1016/j.biotechadv.2014.12.006

PRECISA ENTENDER UM POUCO DE SISTEMA DE RECOMBINAÇÃO



Doi: 10.1016/j.biotechadv.2014.12.006

O PRINCIPIO POR TRÁS DA TÉCNICA...



OPEN ACCESS Freely available online

PLOS ONE

Heritable Genome Editing with CRISPR/Cas9 in the Silkworm, *Bombyx mori*

Wei Wei[1☯], Huhu Xin[2☯], Bhaskar Roy[1], Junbiao Dai[1], Yungen Miao[2*], Guanjun Gao[1*]

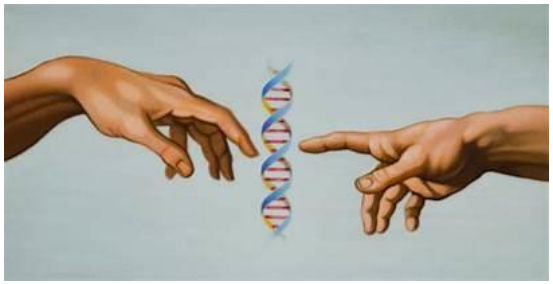
1 School of Life Sciences, Tsinghua University, Beijing, China, **2** College of Animal Sciences, Zhejiang University, Hangzhou, China

Abstract

We report the establishment of an efficient and heritable gene mutagenesis method in the silkworm *Bombyx mori* using modified type II clustered regularly interspaced short palindromic repeats (CRISPR) with an associated protein (Cas9) system. Using four loci *Bm-ok*, *BmKMO*, *BmTH*, and *Bmtan* as candidates, we proved that genome alterations at specific sites could be induced by direct microinjection of specific guide RNA and Cas9-mRNA into silkworm embryos. Mutation frequencies of 16.7–35.0% were observed in the injected generation, and DNA fragments deletions were also noted. *Bm-ok* mosaic mutants were used to test for mutant heritability due to the easily determined translucent epidermal phenotype of *Bm-ok*-disrupted cells. Two crossing strategies were used. In the first, injected *Bm-ok* moths were crossed with wild-type moths, and a 28.6% frequency of germline mutation transmission was observed. In the second strategy, two *Bm-ok* mosaic mutant moths were crossed with each other, and 93.6% of the offsprings appeared mutations in both alleles of *Bm-ok* gene (compound heterozygous). In summary, the CRISPR/Cas9 system can act as a highly specific and heritable gene-editing tool in *Bombyx mori*.

Uso direto do sgRNA e mRNA Cas9!!!!

Bioética??



Protein Cell
DOI 10.1007/s13238-015-0153-5

CrossMark Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

Mol Genet Genomics
DOI 10.1007/s00438-017-1299-z

CrossMark

ORIGINAL ARTICLE

CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein

Lichun Tang[1,2] · Yanting Zeng[3] · Hongzi Du[3] · Mengmeng Gong[1] · Jin Peng[1] · Buxi Zhang[1] · Ming Lei[3] · Fang Zhao[4] · Weihua Wang[5] · Xiaowei Li[6] · Jianqiao Liu[3]

Vídeos

https://www.youtube.com/watch?v=UfA_jAKV29g
https://www.youtube.com/watch?v=TnzcwTyr6cE
https://www.youtube.com/watch?v=jAhjPd4uNFY
https://www.ted.com/talks/jennifer_doudna_we_can_now_edit_our_dna_bu t_let_s_do_it_wisely V

ESTUDO DIRIGIDO

1 Resolução Normativa 16
2. Principio do RNA interferencia (RNAi)
3. Aplicação do RNAi
4. Principio do CRISPR-Cas
5. Aplicação do CRISPR-Cas9

Leitura recomendada

https://innovativegenomics.org/crisprpedia/