LGN5809 – Genética Molécular

CRISPR-CAS – EVOLUÇÃO OU ENGENHARIA GENÉTICA?

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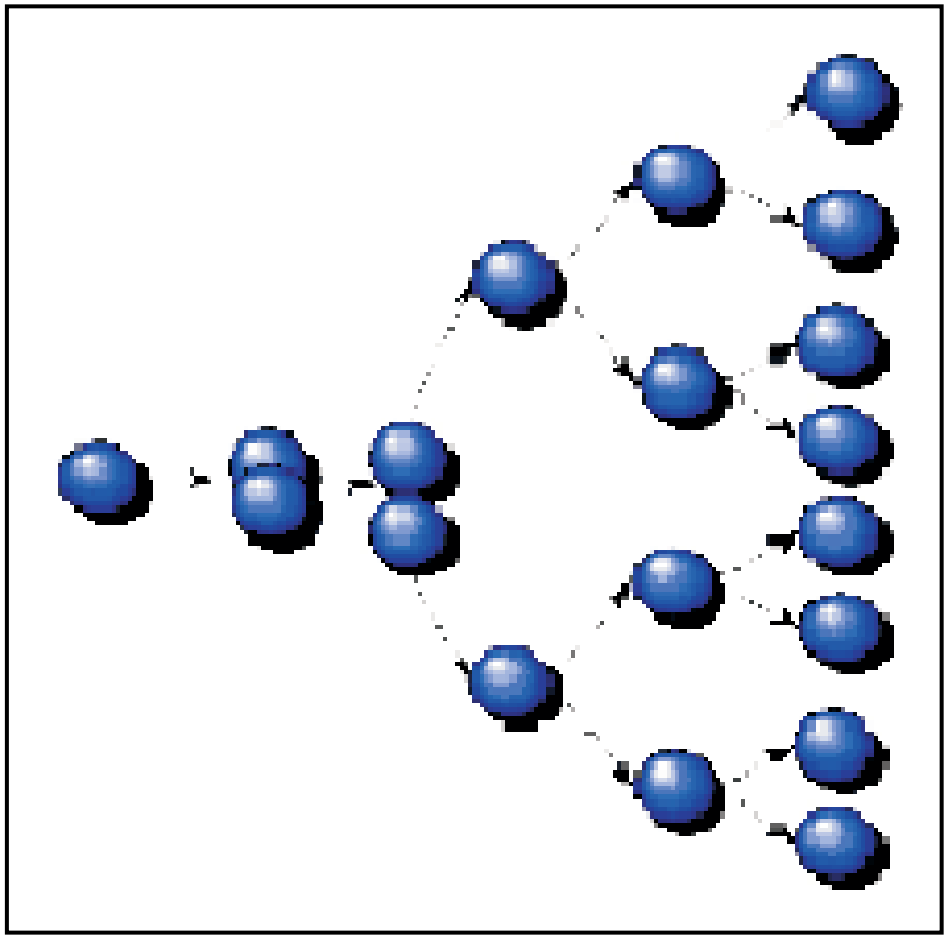
SUMÁRIO

- Fagos e variabilidade em procariotos;
- Definição do Sistema CRISPR-Cas;
- Caracteristicas e histórico das descobertas;
- Mecanismos de ação;
- Aplicações do Sistema CRISPR-Cas;
- Próxima aula

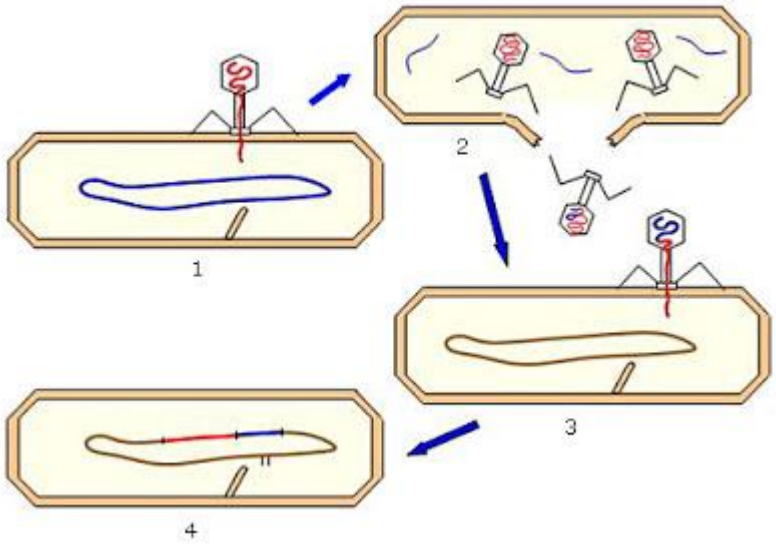
PROCARIOTOS: MODELO CLONAL



Lawrence, 2002.

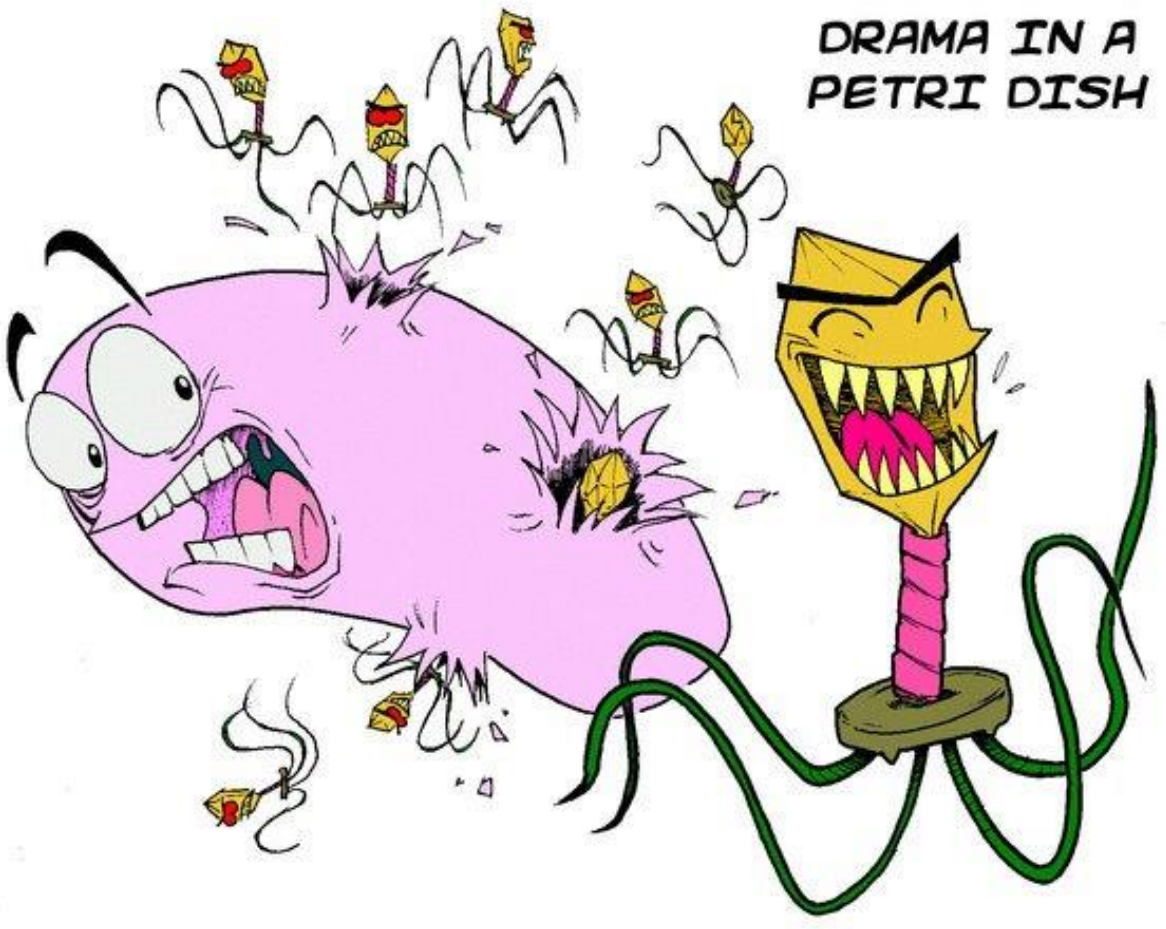
TRANSFERÊNCIA HORIZONTAL DE GENES

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*

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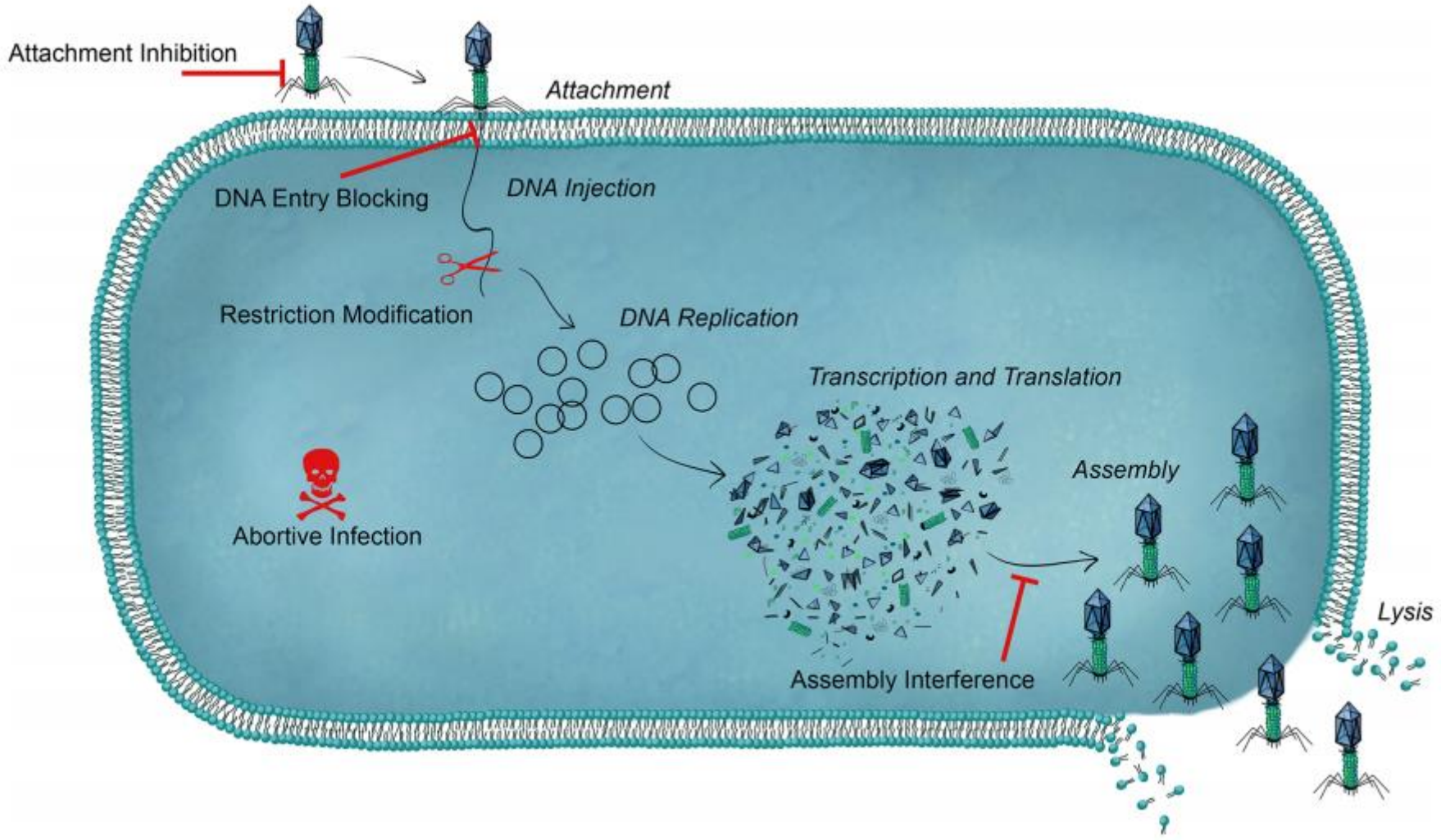
Introduction

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].

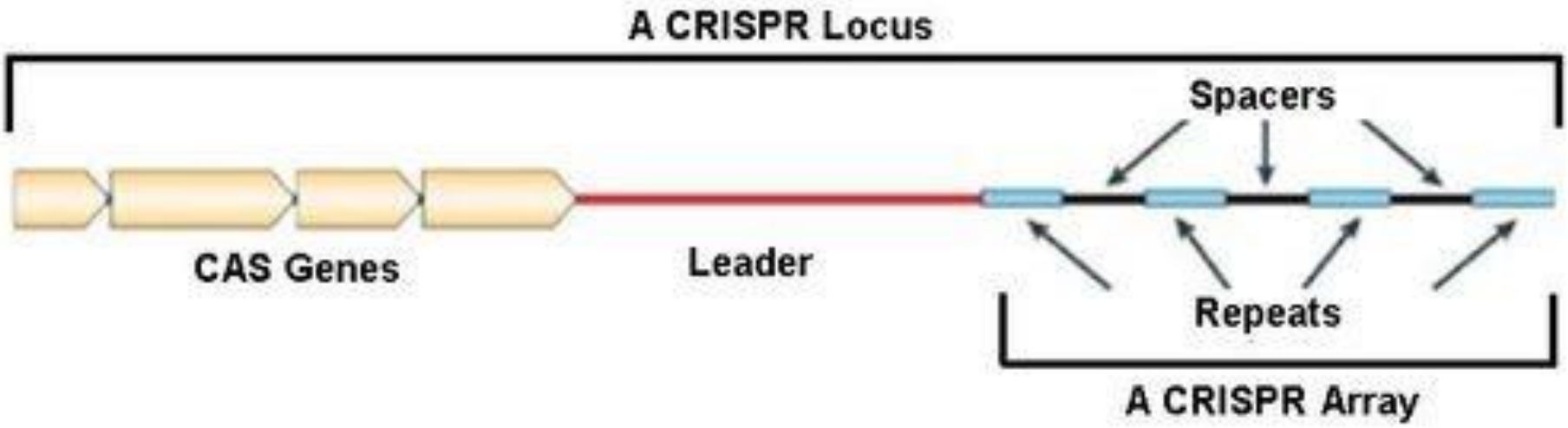
Alguns mecanismos inatos …



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.



Sequencias repetidas de 24 a 47 pb, com espaçadores de of 26–72 pb

Espaçadores - 75% sequencias fagos e 20% plasmideos

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”

No começo

Table 1. Main features of the SRSRs.

Organism	SRSR size (bp)	Spacing (bp)	Number of clusters	SRSR units per cluster	Reference
Archaea					
H. volcanii	30	ND	≥2	ND	Mojica *et al.* (1995) *Mol Microbiol* **9**: 13–21
H. mediterranei	30	33–39	3	21/ ND / ND	Mojica *et al.* (1995) *Mol Microbiol* **9**: 13–21
M. jannaschii	28–30	31–51	$7^{A} + 6^{B} + 1^{C}$	4–25	Bult *et al.* (1996) *Science* **273**: 1058–1073 and this work
M.					
A.					
S.					
P.					
P.					
A.					
Ba					
T.					
A. aeolicus	29	36–38	1	6	This work
E. coli	29	32–33	3	2/7/13	Nakata *et al.* (1989) *J Bacteriol* **171**: 3553–3556 and this work
S. typhi	29	32	≥1	6	This work
C. jejuni	36	30	1	5	This work
Y. pestis	28	32–33	2	6/9	This work
C. difficile	29	36–38	$4^{A} + 2^{B}$	5–17	This work
M. tuberculosis	36	38–40	1	Variable	Hermans *et al.* (1991) *Infect Immun* **59**: 2695–705
Calothrix sp.	37	35–41	>1	5	Masepohl *et al.* (1996) *Biochim Biophys Acta* **1307**: 20–36
Anabaena sp.	37	32–43	>1	17	Masepohl *et al.* (1996) *Biochim Biophys Acta* **1307**: 20–36
Mitochondria					
V. faba	40	20–35	1	6	Flamand *et al.* (1992) *Plant Mol Biol* **19**: 913–923

A,B, Types of SRSRs distinct (more than 3 bp differences) within the same microorganism. ND, Not determined.

Recordista: *Verminephrobacter eiseniae*, possui 249 *repeats per array*

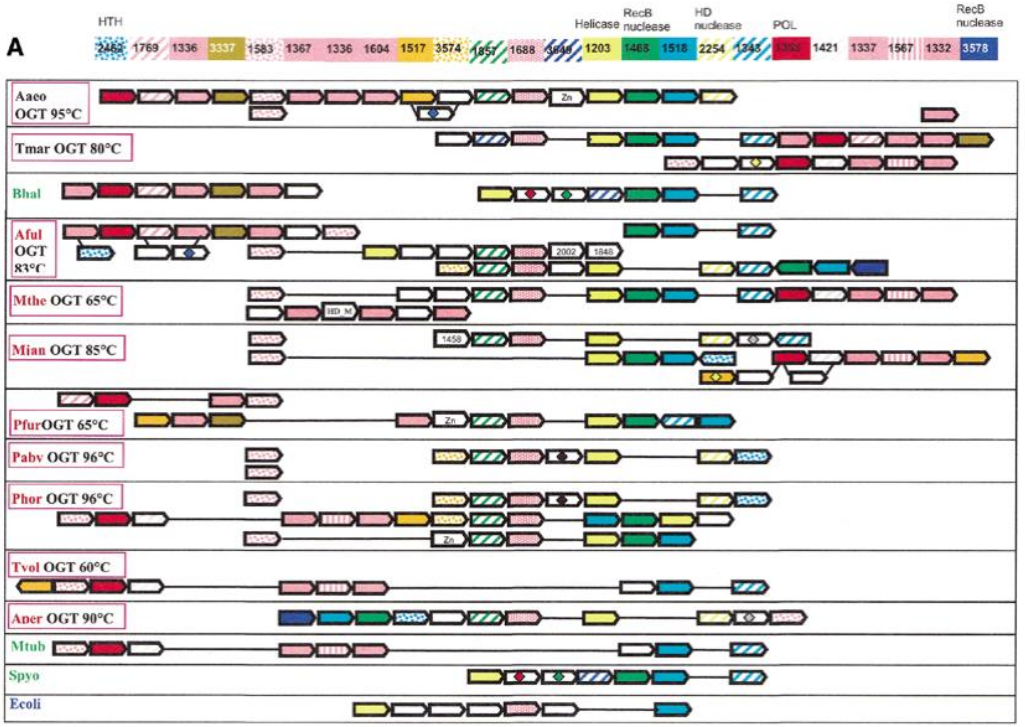
Mol. Microbiol. 2000; 36:244–246.

482–496 *Nucleic Acids Research, 2002, Vol. 30, No. 2*

A DNA repair system specific for thermophilic Archaea and bacteria predicted by genomic context analysis

Kira S. Makarova[1,2], L. Aravind[1], Nick V. Grishin[3], Igor B. Rogozin[1] and Eugene V. Koonin[1,*]

Sistema de reparo de DNA?



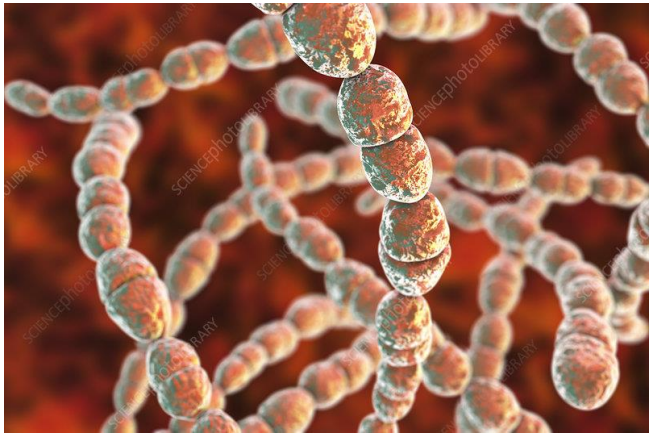
Na realidade ....

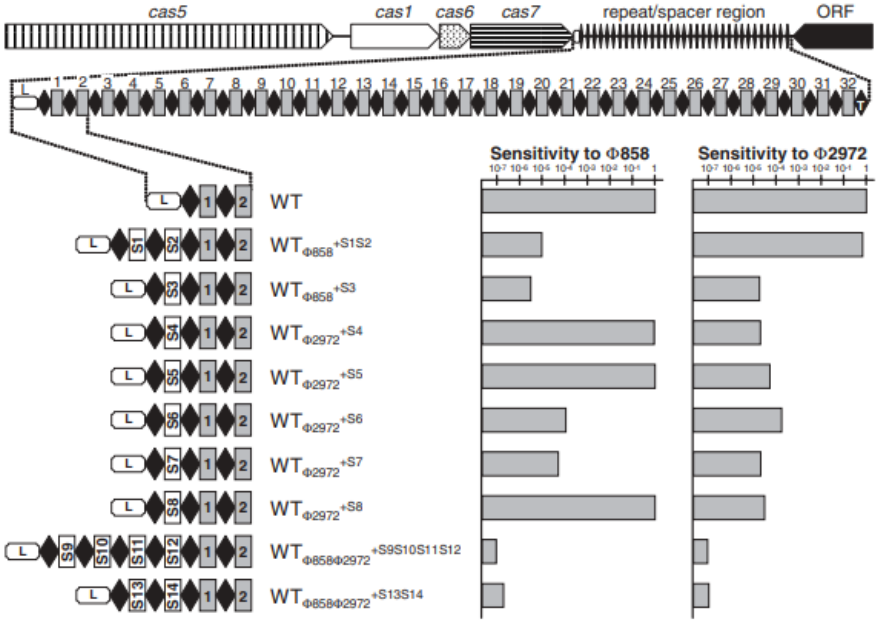
CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

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]*

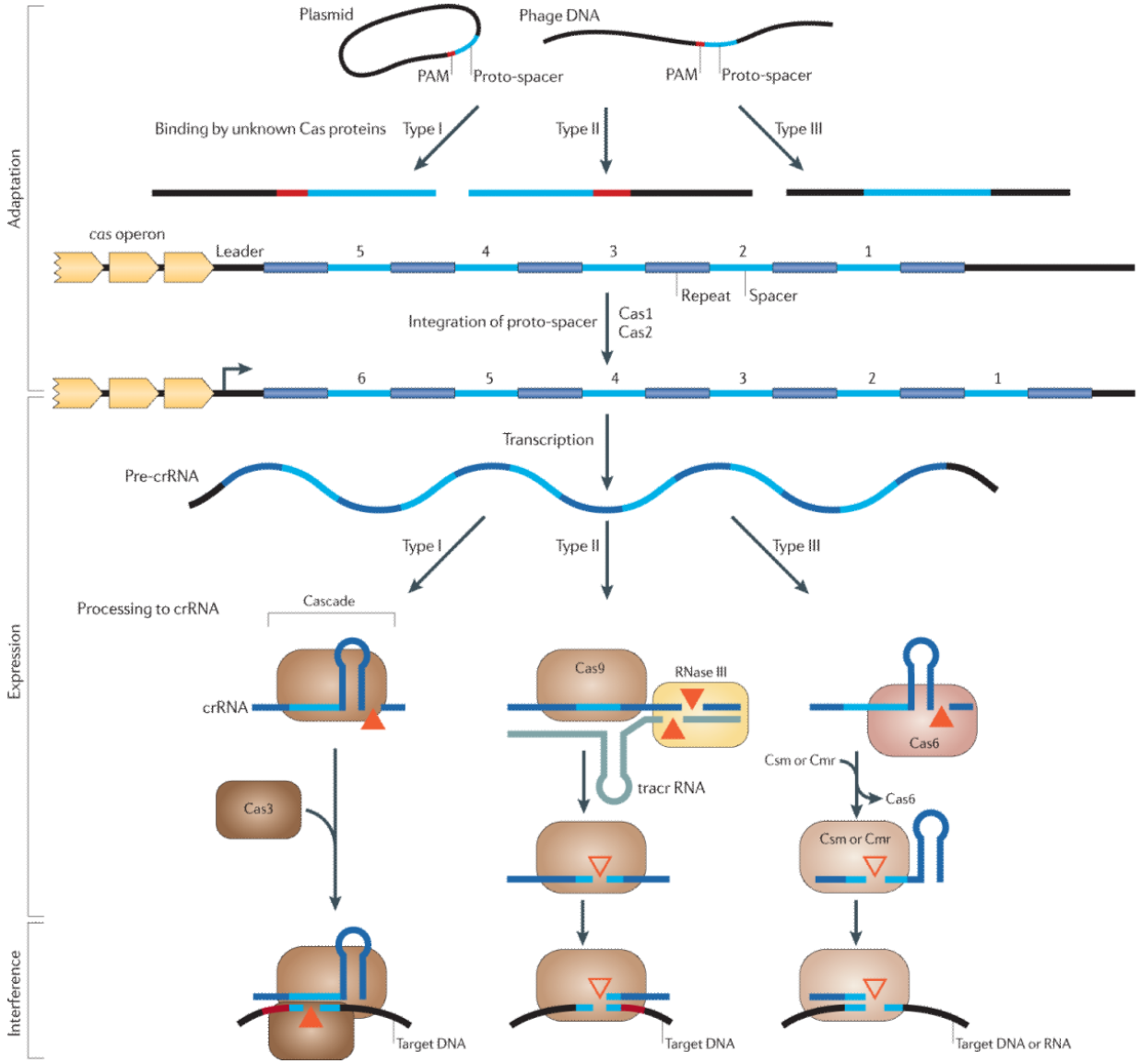
Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences.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





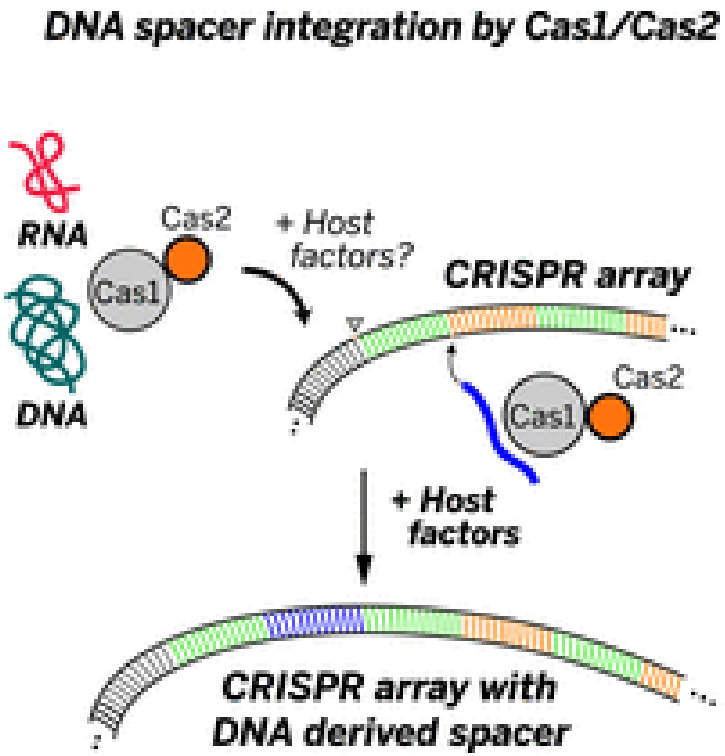
Três estágios da ação do CRISPR–Cas

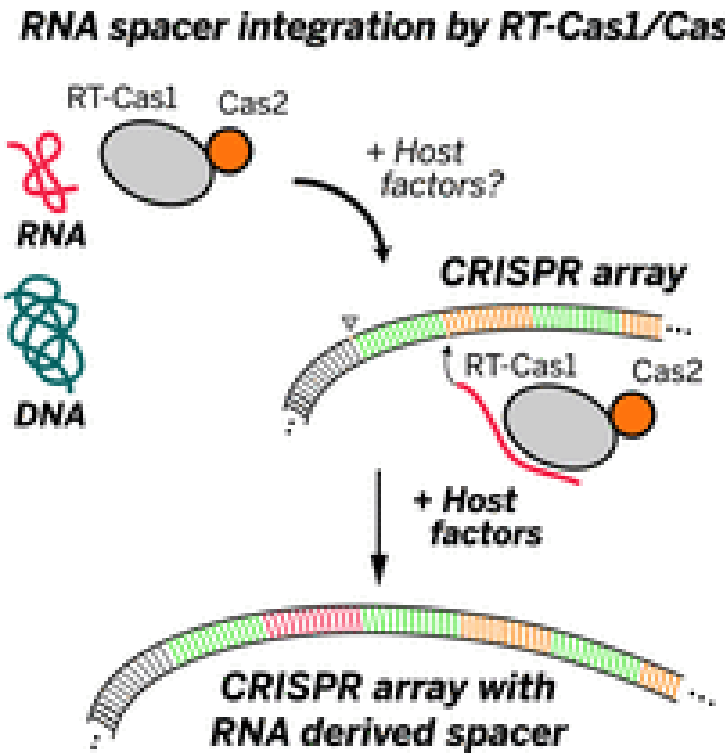


AS PROTEÍNAS CAS NÃO SÃO CONSERVADAS

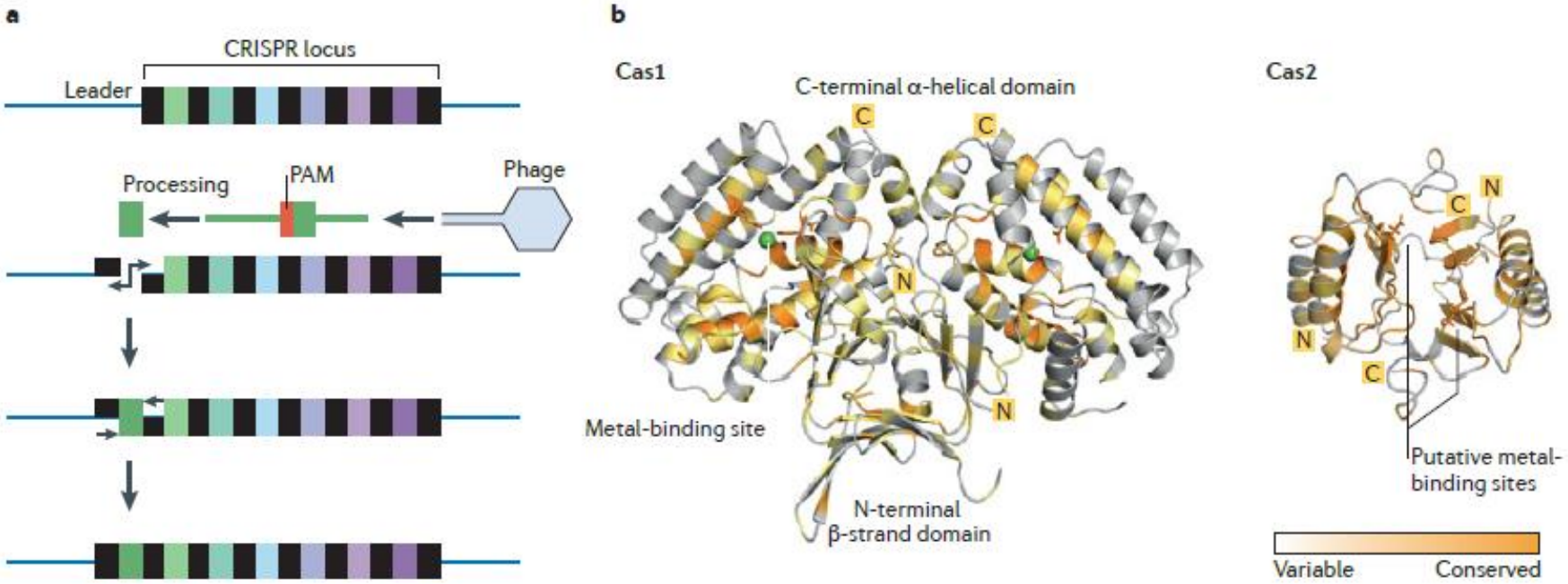
Cas1: metal-dependent Dnase que não tem especificidade à sequencias e tem sido proposto estar envolvida na integração do “spacer DNA” no cassete CRISPR,

Cas2: metal-dependent endoribonuclease cujo mecanismo no Sistema CRISPR–Cas continua obscuro





Aquisição do “CRISPR SPACER”



ALTA VARIAÇÃO NAS ETAPAS POSTERIORES...

Um complexo enzimático – endoribonucleases (**Cascade** - CRISPR-associated complex for antiviral defence)

Cas 6 para arquéias - *Pyrococcus furiosus*

Em *E. coli*, a clivagem provavelmente é catalisada por um domínio HD endonuclease da proteína Cas3

in vitro experimentos com o sistema CRISPR–Cas proveniente de *P. furiosus* demonstrou que nessa espécie o sistema CRISPR degrada mRNA

Cell

RNA-Guided RNA Cleavage by a CRISPR RNA-Cas Protein Complex

Caryn R. Hale,[1] Peng Zhao,[1] Sara Olson,[3] Michael O. Duff,[3] Brenton R. Graveley,[3] Lance Wells,[1] Rebecca M. Terns,[1,*] and Michael P. Terns[1,2,*]
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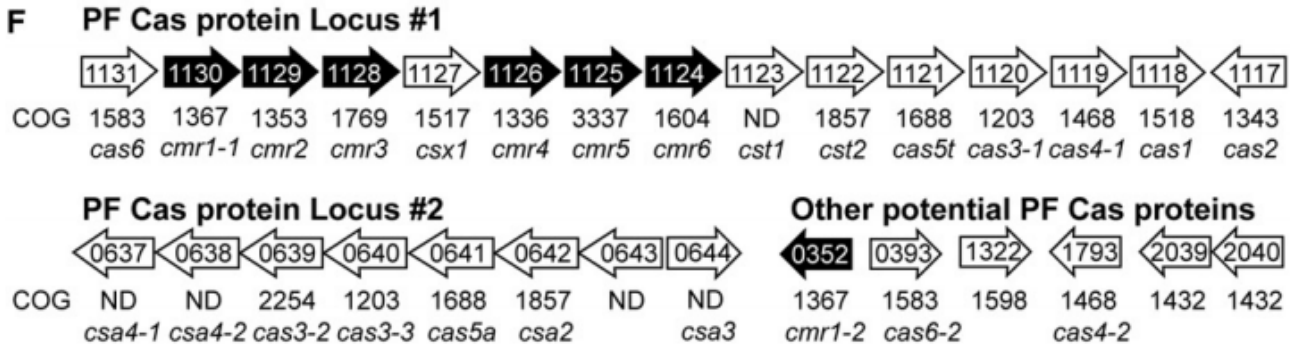
*Correspondence: rterns@bmb.uga.edu (R.M.T.), mterns@bmb.uga.edu (M.P.T.)
DOI 10.1016/j.cell.2009.07.040



COMO DETECTAR O CRISPR CAS EM GENOMAS?

Table 1 | **Web resources for CRISPR analysis**

Resource and web page	Description	Refs
PILER-CR; http://www.drive5.com/pilercr/	A software tool for the detection of CRISPRs in microbial genomic sequences; based on local alignments in the genome that are represented by mathematical graphs*	49
CRISPR Recognition Tool; http://www.room220.com/crt/	A software tool for the detection of CRISPRs in microbial genomic sequences; based on the detection of exact k-mer matches that are separated by similar distances*	50
CRISPRFinder; http://crispr.u-psud.fr/crispr/	A software tool for the detection of CRISPRs in microbial genomic sequences; based on enhanced suffix arrays*	51
CRISPRdb; http://crispr.u-psud.fr/crispr/	Automatically updated database of CRISPR arrays in published microbial genomes; also contains CRISPR analysis tools that allow the alignment and comparison of repeats and spacers against the public databases	12
Pygram; http://www.irisa.fr/symbiose/projets/Modulome/article.php3?id_article=18	Visualization application that provides a graphical browser for studying repeats	52
TIGR Comprehensive Microbial Resource; http://rice.tigr.org/tigr-scripts/CMR2/genome_property.spl?subproperty=CRISPR%20region!&select_count=1	Provides a 'clickable' table that depicts, for each sequenced genome, the presence or absence of the 45 Cas protein families that are defined in Ref. 17	17

*This CRISPR (clustered, regularly interspaced short palindromic repeat) detection software applies post-processing filters to separate real CRISPR arrays from false predictions. BLAST, Basic Local Alignment Search Tool.

doi:10.1038/nrmicro1793

BMC Bioinformatics

BioMed Central

Database

Open Access

The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats

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Address: [1]Univ Paris-Sud, Institut de Génétique et Microbiologie, UMR 8621, Orsay, F-91405, France; CNRS, Orsay, F-91405, France and [2]Centre d'Etudes du Bouchet, 5 rue Lavoisier, 91710 Vert le Petit, France

Email: Ibtissem Grissa - ibtissem.grissa@igmors.u-psud.fr; Gilles Vergnaud - gilles.vergnaud@igmors.u-psud.fr; Christine Pourcel* - christine.pourcel@igmors.u-psud.fr

* Corresponding author

OXFORD (GIGA)n SCIENCE

GigaScience, 9, 2020, 1–12

doi: 10.1093/gigascience/giaa062

Technical Note

TECHNICAL NOTE

CRISPRcasIdentifier: Machine learning for accurate identification and classification of CRISPR-Cas systems

Victor A. Padilha[1,†], Omer S. Alkhnbashi[2,†], Shiraz A. Shah[3], André C. P. L. F. de Carvalho[1] and Rolf Backofen[2,4,*]

CLASSIFICAÇÃO DE CRISPR CAS...

OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

A Guild of 45 CRISPR-Associated (Cas) Protein Families and Multiple CRISPR/Cas Subtypes Exist in Prokaryotic Genomes

Daniel H. Haft*, Jeremy Selengut, Emmanuel F. Mongodin, Karen E. Nelson

The Institute for Genomic Research, Rockville, Maryland, United States of America

Clustered regularly interspaced short palindromic repeats (CRISPRs) are a family of DNA direct repeats found in many prokaryotic genomes. Repeats of 21–37 bp typically show weak dyad symmetry and are separated by regularly sized, nonrepetitive spacer sequences. Four CRISPR-associated (Cas) protein families, designated Cas1 to Cas4, are strictly associated with CRISPR elements and always occur near a repeat cluster. Some spacers originate from mobile genetic elements and are thought to confer "immunity" against the elements that harbor these sequences. In the present study, we have systematically investigated uncharacterized proteins encoded in the vicinity of these CRISPRs and found many additional protein families that are strictly associated with CRISPR loci across multiple prokaryotic species. Multiple sequence alignments and hidden Markov models have been built for 45 Cas protein families. These models identify family members with high sensitivity and selectivity and classify key regulators of development, DevR and DevS, in *Myxococcus xanthus* as Cas proteins. These identifications show that CRISPR/*cas* gene regions can be quite large, with up to 20 different, tandem-arranged *cas* genes next to a repeat cluster or filling the region between two repeat clusters. Distinctive subsets of the collection of Cas proteins recur in phylogenetically distant species and correlate with characteristic repeat periodicity. The analyses presented here support initial proposals of mobility of these units, along with the likelihood that loci of different subtypes interact with one another as well as with host cell defensive, replicative, and regulatory systems. It is evident from this analysis that CRISPR/*cas* loci are larger, more complex, and more heterogeneous than previously appreciated.

Citation: Haft DH, Selengut J, Mongodin EF, Nelson KE (2005) A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS Comput Biol 1(6): e60.

DOI: 10.1371/journal.pcbi.0010060

Table 1. Description of the Different *cas* Core Genes, CRISPR/Cas Subtypes, and the RAMP Module, Based on the New Cas Protein Families

Category	Gene	Example Locus	Specific HMM	COG	Putative Function/Family	Notes
Core proteins	*cas1*	AF1878	TIGR00287	COG1518	Putative novel nuclease [a]	—
	cas2	AF1876	TIGR01573	COG1343, COG3512	—	—
		CT1918	TIGR01873	COG1343	—	Ecoli subtype-specific
	cas3	AF1874	TIGR01587	COG1203	Helicase (PF00271)	Core domain
		AF1875	TIGR01596	COG2254	Nuclease (PF01966)	HD domain
		YPO2467	TIGR02562	COG1203	Helicase (PF00271)	Ypest subtype-specific
	cas4	AF1877	TIGR00372	COG1468	RecB-family exonuclease [a,b]	—
	cas5	AF1872	TIGR02593	—	—	N-terminal domain
	cas6	AF1859	TIGR01877	COG1583	Possible RAMP [a]	When present, usually first
Ecoli subtype	*cse1*	CT1972	TIGR02547	—	—	—
	cse2	CT1973	TIGR02548	—	—	—
	cse3	CT1974	TIGR01907	—	—	—
	cse4	CT1975	TIGR01869	—	—	—
	cas5e	CT1976	TIGR01868	—	Cas5 N-terminal domain	—
Ypest subtype	*csy1*	YPO2465	TIGR02564	—	—	—
	csy2	YPO2464	TIGR02565	—	—	—
	csy3	YPO2463	TIGR02566	—	—	—
	csy4	YPO2462	TIGR02563	—	—	—
Nmeni subtype	*csn1*	SPs1176	TIGR01865	COG3513	HNH endonuclease?	—
	csn2	SPs1173	TIGR01866	—	—	Not always present
Dvulg subtype	*csd1*	CT1133	TIGR01863	—	—	—
	csd2	CT1132	TIGR02589	COG3649	—	—
	cas5d	CT1134	TIGR01876	—	Cas5 N-terminal domain	—
Tneap subtype	*cst1*	GTN1972	TIGR01908	—	Contains CXXC–CXXC motif	Occasionally absent
	cst2	GTN1971	TIGR02585	COG1857	Regulator (TIGR01875)	Related to Csa2
	cas5t	GTN1970	TIGR01895	COG1688	Cas5 N-terminal domain	—
Hmari subtype	*csh1*	TM1802	TIGR02591	—	Often contains CXXC–CXXC motif	—
	csh2	TM1801	TIGR02590	COG3649	Regulator (TIGR01875)	Related to Csd2
	cas5h	TM1800	TIGR02592	COG1688	Cas5 N-terminal domain	—
Apern subtype	*csa1*	AF1879	TIGR01896	COG4343	—	Usually proximal to repeat
	csa2	AF1871	TIGR02583	COG1857	Regulator (TIGR01875)	—
	csa3	AF1869	TIGR01884	COG0640	Helix-turn-helix, transcriptional regulator	Distantly related to PF01022
	csa4	MJ0385	TIGR01914	—	—	Occasionally absent
	csa5	AF1870	TIGR01878	—	—	Occasionally absent
	cas5a	AF1872	TIGR01874	COG1688	Cas5 N-terminal domain	—
Mtube subtype	*csm1*	TM1811	TIGR02578	COG1353	Putative novel polymerase [a]	Related to Cmr2
	csm2	TM1810	TIGR01870	COG1421	—	—
	csm3	TM1809	TIGR02582	COG1337	RAMP (PF03787)	Related to Cmr4
	csm4	TM1808	TIGR01903	COG1567	RAMP (PF03787)	—
	csm5	TM1807	TIGR01899	COG1332	RAMP (PF03787)	—
RAMP module	*cmr1*	TM1795	TIGR01894	COG1367	RAMP (PF03787)	—
	cmr2	TM1794	TIGR02577	COG1353	Putative novel polymerase[a]	Related to Csm1
	cmr3	TM1793	TIGR01888	COG1769	RAMP [a]	—
	cmr4	TM1792	TIGR02580	COG1336	RAMP (PF03787)	Related to Csm3
	cmr5	TM1791.1	TIGR01881	COG3337	—	—
	cmr6	TM1791	TIGR01898	COG1604	RAMP (PF03787)	—

[a]Makarova et al. [14].
[b]Jansen et al.[3].
DOI: 10.1371/journal.pcbi.0010060.t001

DOI: 10.1371/journal.pcbi.0010060

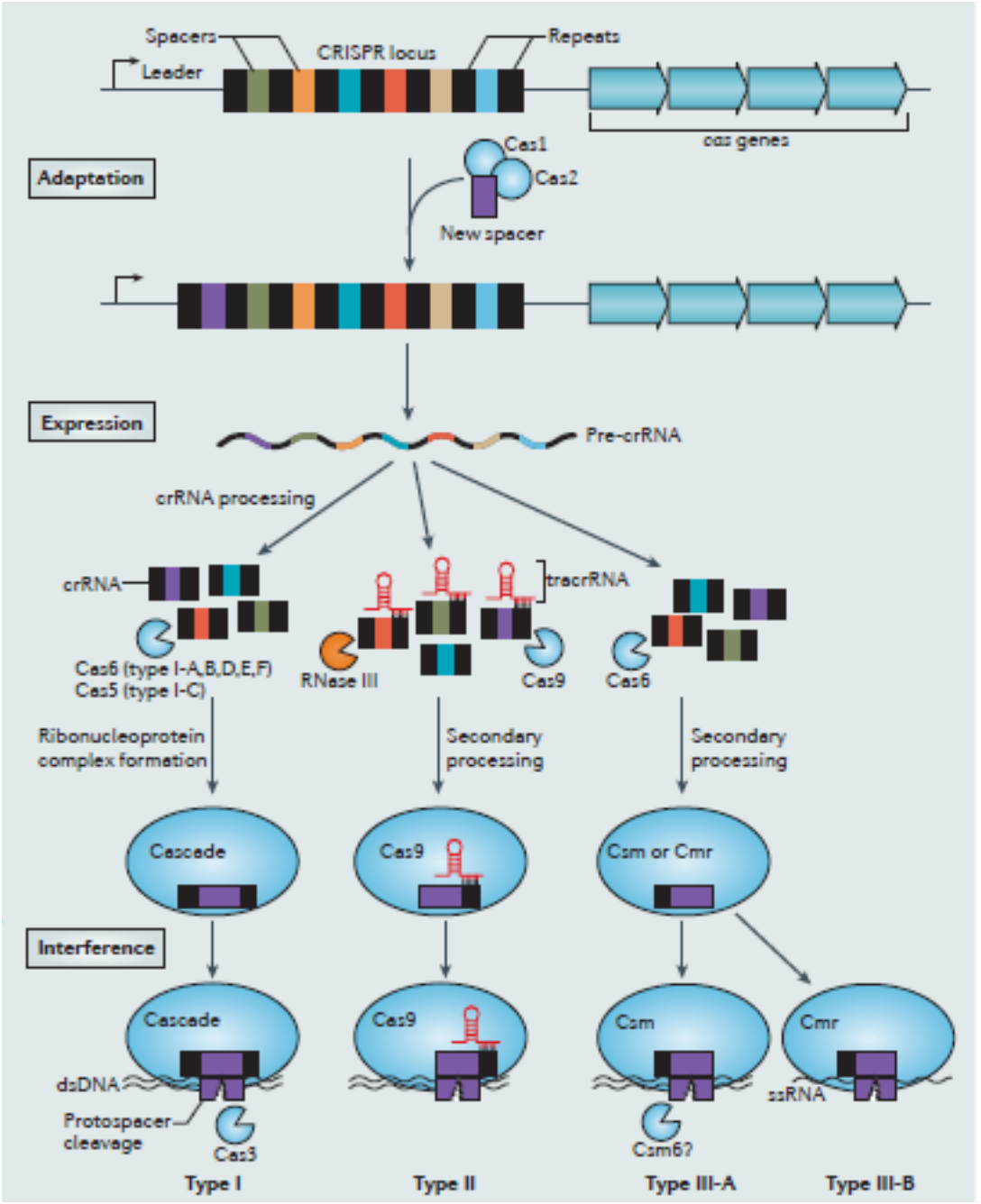
Alguns problemas :

Multiplos sistemas CRISPR–Cas em um mesmo genoma

Inconsistência com a classificação

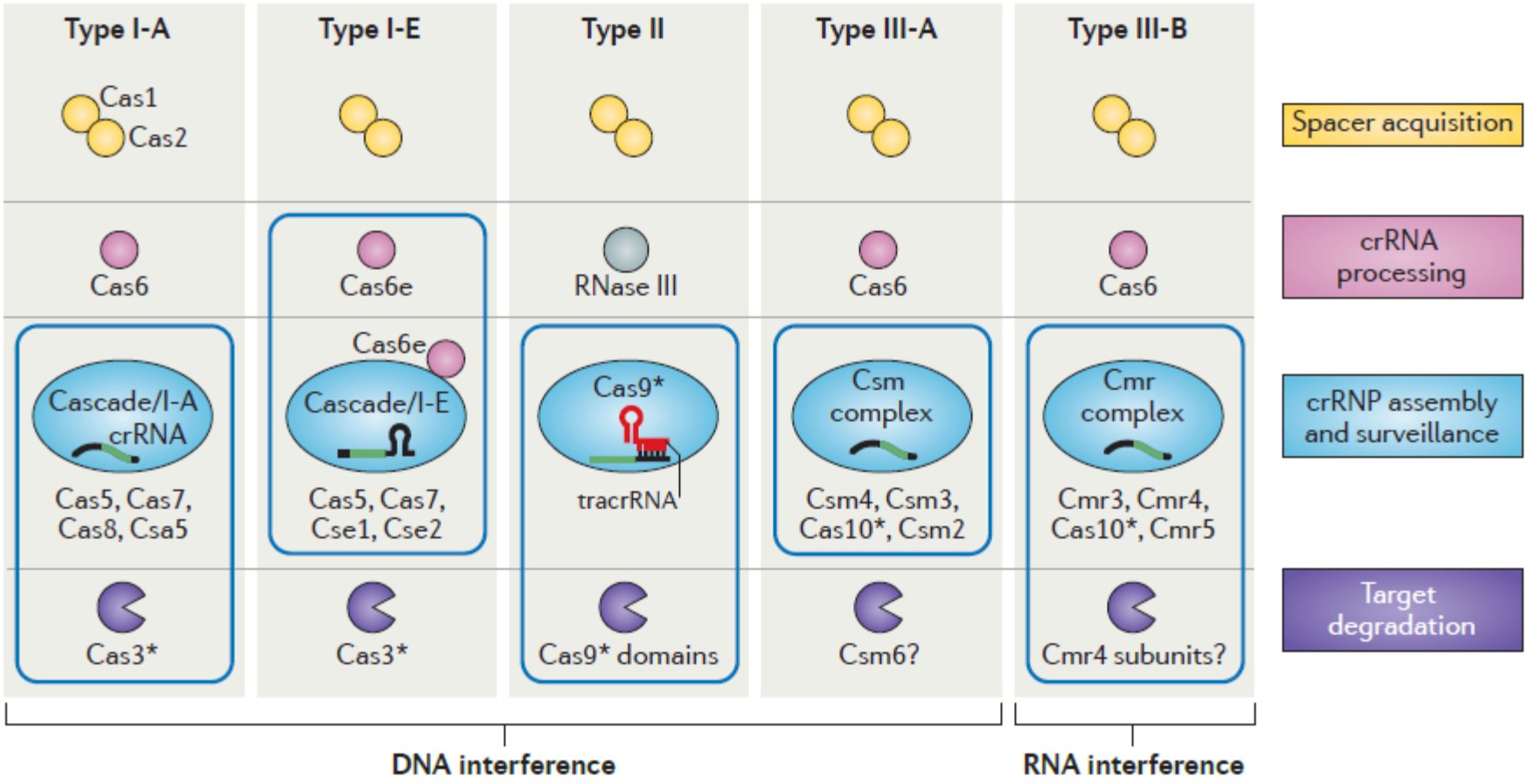
DOI: 10.1371/journal.pcbi.0010060

Classes de CRISPR-Cas

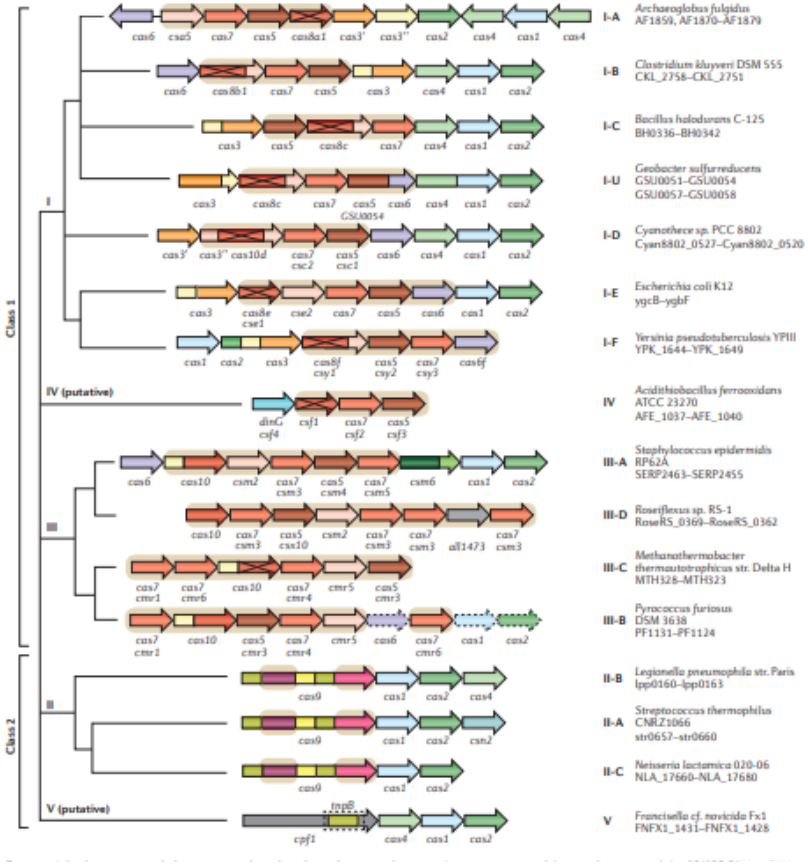


doi:10.1038/nrmicro3241

TIPOS DE CRISPR CAS – PANORAMA GERAL

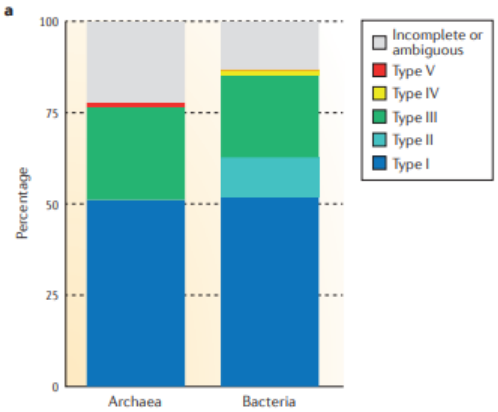


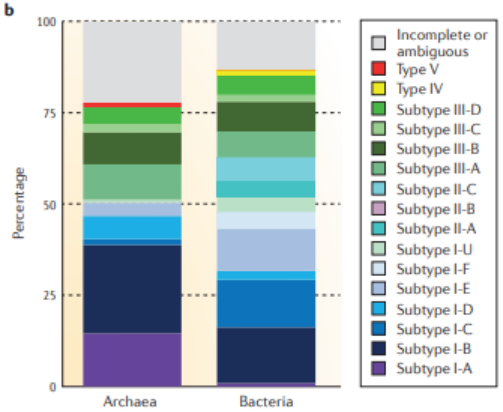
doi:10.1038/nrmicro3279



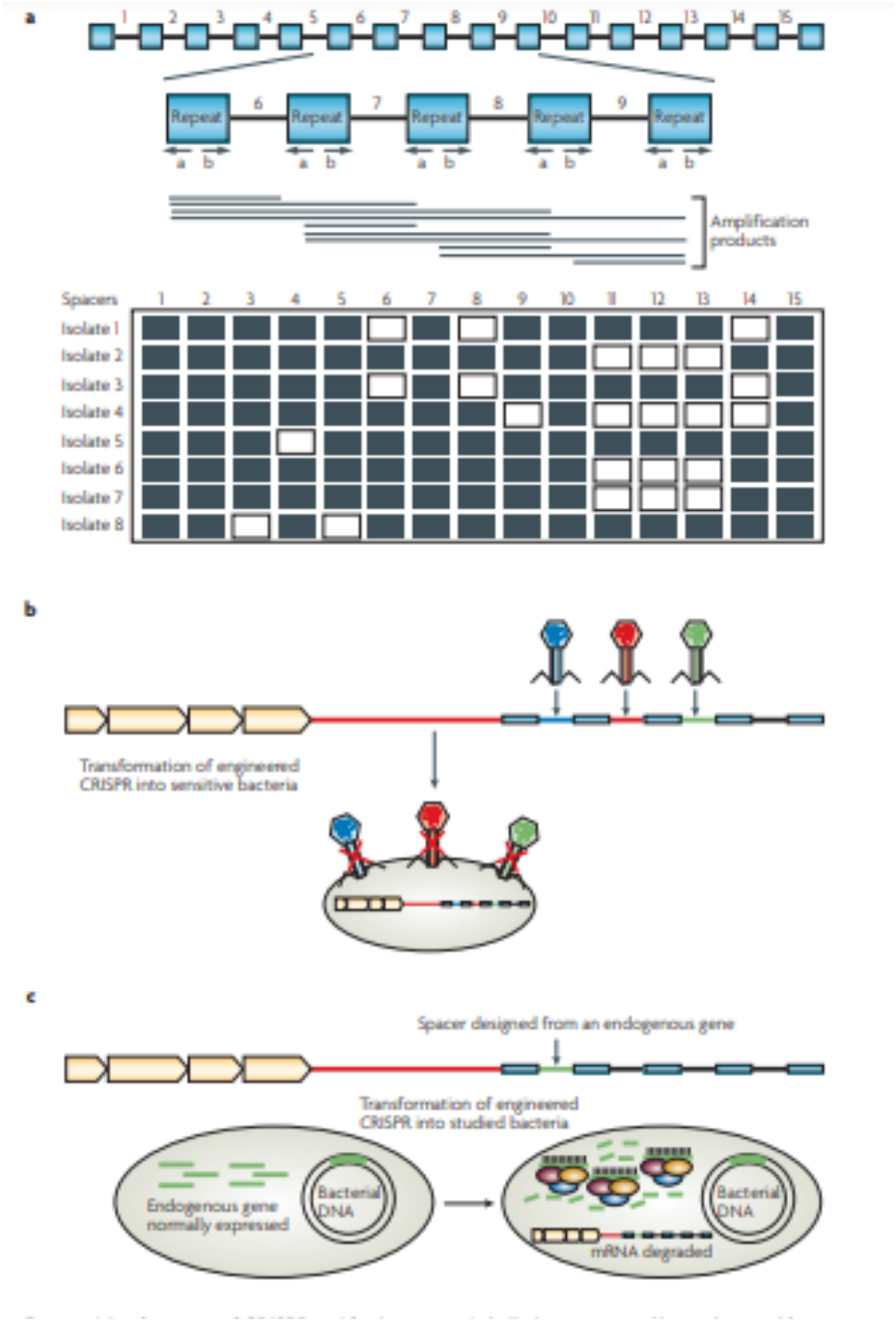
An updated evolutionary classification of CRISPR–Cas systems

Kira S. Makarova[1], Yuri I. Wolf[1], Omer S. Alkhnbashi[2], Fabrizio Costa[2], Shiraz A. Shah[3], Sita J. Saunders[2], Rodolphe Barrangou[4], Stan J. J. Brouns[5], Emmanuelle Charpentier[6], Daniel H. Haft[1], Philippe Horvath[7], Sylvain Moineau[8], Francisco J. M. Mojica[9], Rebecca M. Terns[10], Michael P. Terns[10], Malcolm F. White[11], Alexander F. Yakunin[12], Roger A. Garrett[3], John van der Oost[5], Rolf Backofen[2,13] and Eugene V. Koonin[1]





COMO USAR ISSO EM MEU LABORATÓRIO?



Em 2008 – previsão de uso do Sistema CRISPR-Cas

UM DOS PRIMEIROS TRABALHOS…

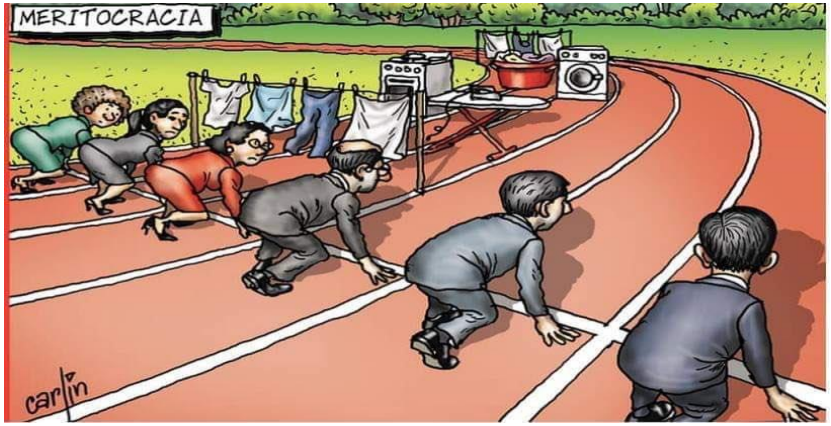
A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

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….

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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,*]

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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.

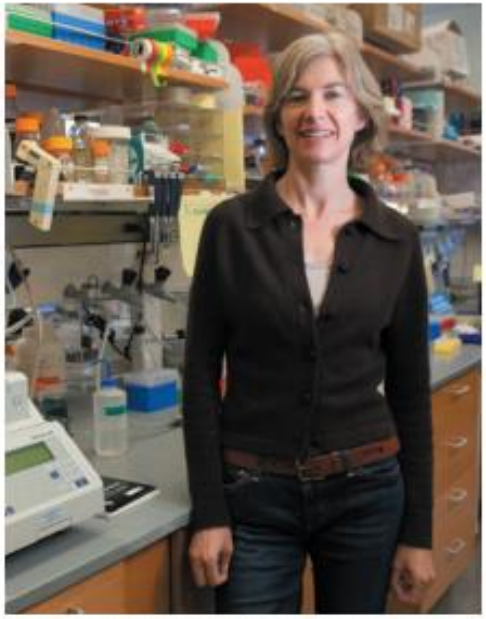
BY HEIDI LEDFORD

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

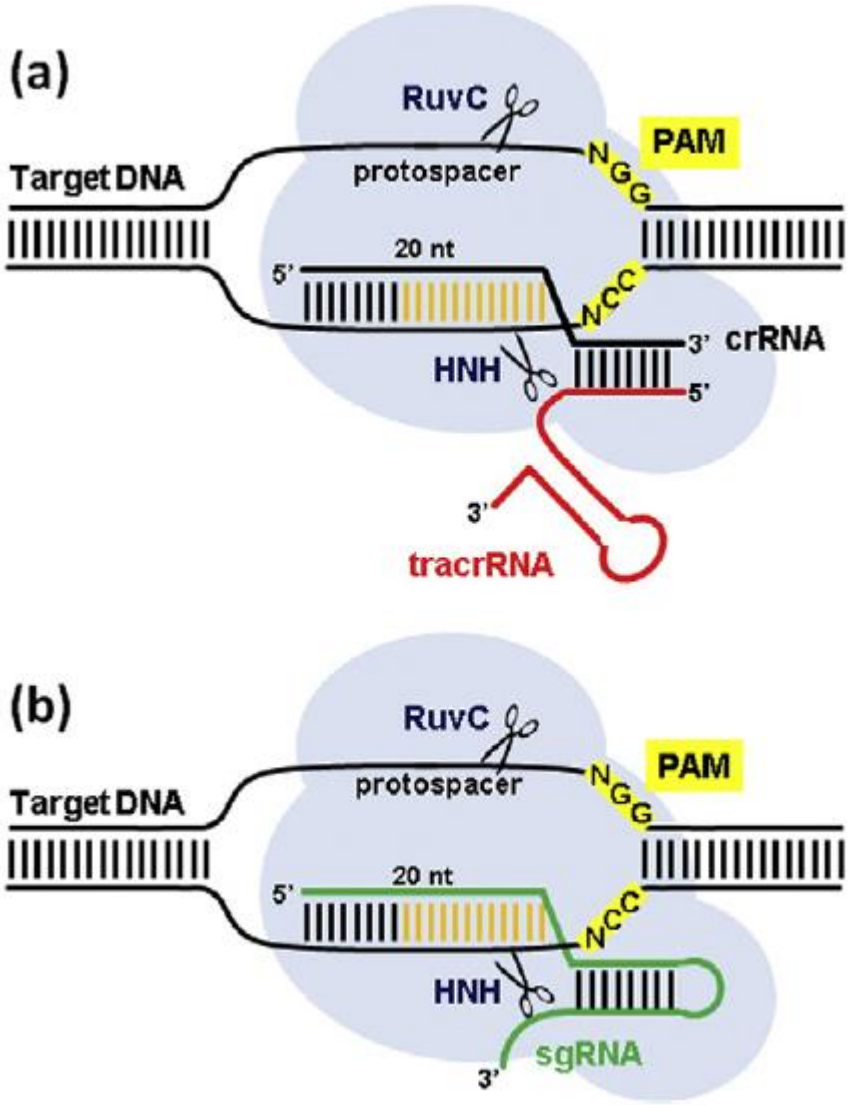
STAT+

CRISPR patent agreement seeks to expand use in crops

By SHARON BEGLEY @sxbegle / OCTOBER 18, 2017

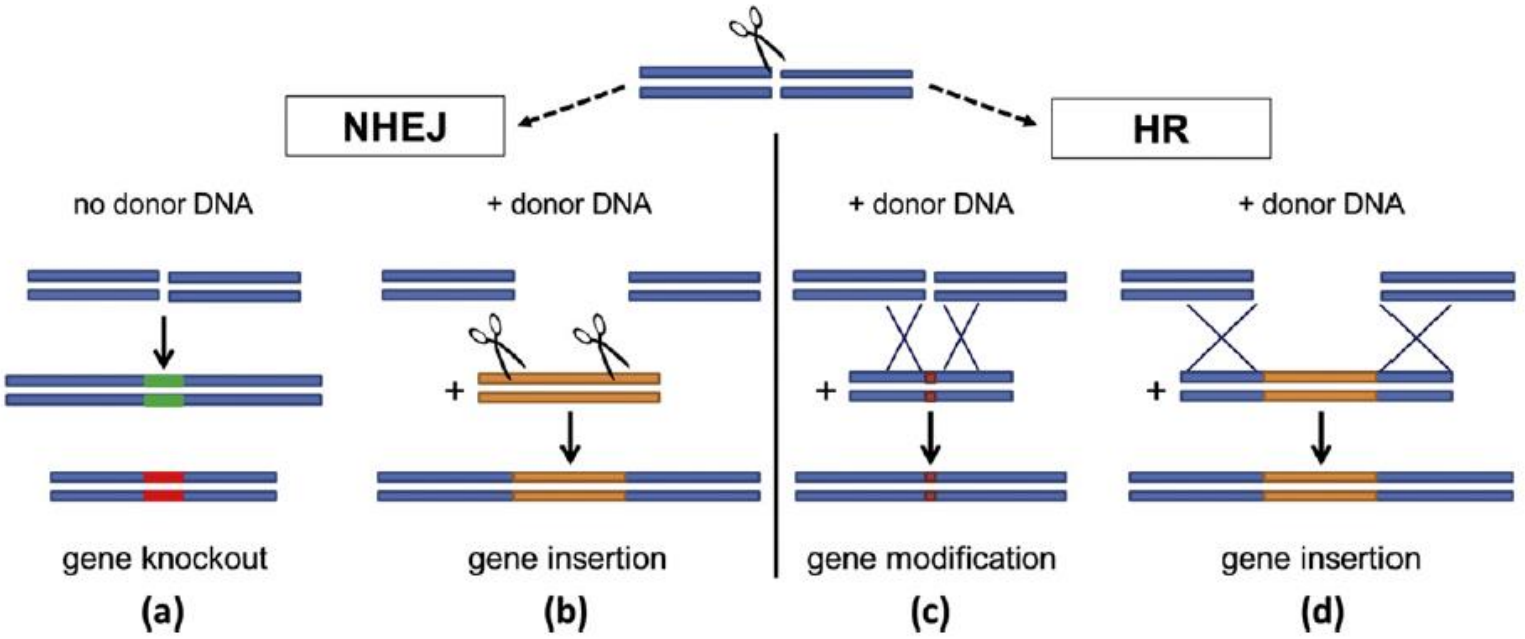
https://www.statnews.com/2017/10/18/crispr-patent-expand-crops/

Otimização do Sistema Crispr-Cas9



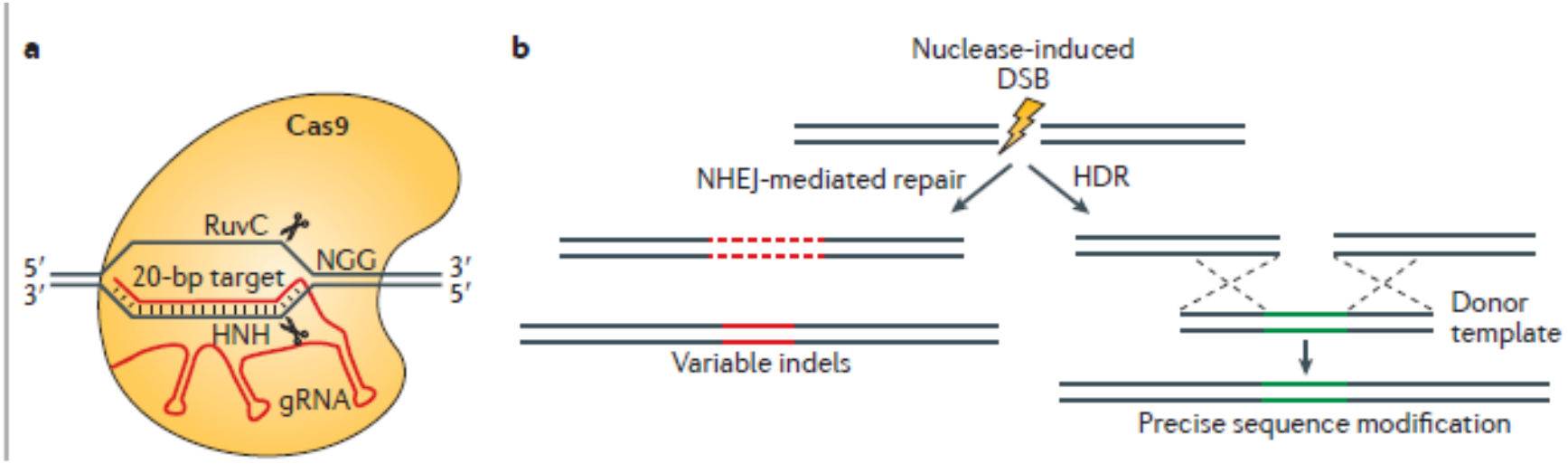
doi: 10.1016/j.biotechadv.2014.12.006

PRECISA ENTENDER UM POUCO DE SISTEMA DE RECOMBINAÇÃO



Doi: 10.1016/j.biotechadv.2014.12.006

O PRINCIPIO POR TRÁS DA TÉCNICA...



Depois expandiu para todos os reinos…

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CRISPR-assisted editing of bacterial genomes

Wenyan Jiang[1,4], **David Bikard**[1,4], **David Cox**[2,3], **Feng Zhang**[2,3], and **Luciano A. Marraffini**[1]

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[2]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA.

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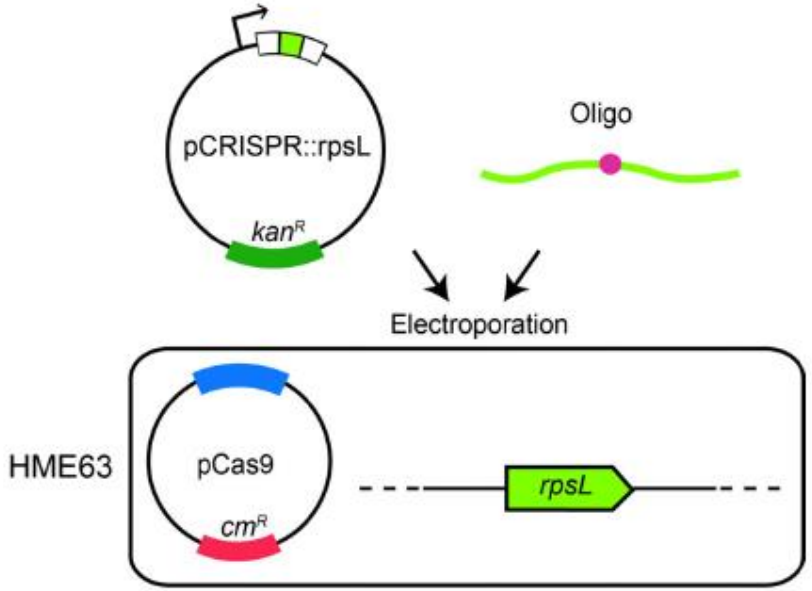

Agora…vários protocolos e kits

STUDY DESIGNS

Am I ready for CRISPR? A user's guide to genetic screens

John G. Doench

Abstract | Exciting new technologies are often self-limiting in their rollout, as access to state-of-the-art instrumentation or the need for years of hands-on experience, for better or worse, ensures slow adoption by the community. CRISPR technology, however, presents the opposite dilemma, where the simplicity of the system enabled the parallel development of many applications, improvements and derivatives, and new users are now presented with an almost paralyzing abundance of choices. This Review intends to guide users through the process of applying CRISPR technology to their biological problems of interest, especially in the context of discovering gene function at scale.

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

The CRISPR/Cas9 system for plant genome editing and beyond

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[a] Institute for Molecular Biotechnology, RWTH Aachen University, Aachen, Germany

Table 2
CRISPR-Cas9 mediated NHEJ in stable transformants.

Species	Transformation method	Cas9 codon optimization	Promoters (Cas9, gRNA)	Target	Mutation frequency	Detection method	Off-target/detection method	Multiplex (deletion)	Transmission to progeny	Reference
Arabidopsis thaliana	Agro-transformation by floral dip	Human	AtUBQ1, AtU6	Co-transfected *GUUS*	80% (35/44)	Surveyor assay				Mao et al. (2013)
A. thaliana	Agro-transformation by floral dip	Human	AtUBQ1, AtU6	*TT4, CHL1, CHL12*	38–89%	Phenotype, surveyor assay, sequencing		Yes (230 bp)		Mao et al. (2013)
A. thaliana	Agro-transformation by floral dip	Arabidopsis (Nickase)	PcUBI4-2	*ADH1, TT4, RTEL1*	0.04%	PCR + deep sequencing				Fauser et al. (2014)
A. thaliana	Agro-transformation by floral dip	Arabidopsis	PcUBI4-2	*ADH1, TT4, RTEL1*	26.7%	PCR + deep sequencing			Yes	Fauser et al. (2014)
A. thaliana	Agro-transformation by floral dip	Arabidopsis (Nickase)	PcUBI4-2	*ADH1*	42.8% (Paired Nickases)	PCR + deep sequencing			Yes	Schiml et al. (2014)
A. thaliana	Agro-transformation by floral dip	*C. reinhardtii*	CaMV35S, AtU6	co-transfected *GFP*	58% (35/60)	GFP fluorescence			Yes	Jia et al. (2014)
A. thaliana	Agro-transformation by floral dip	Human	2xCaMV35S, AtU6	*BRI1, GAI, JAZ1*	30% (6/20)–84%(16/19)	PCR + RE			Yes	Feng et al. (2013)
Oryza sativa	Particle bombardment of callus	Rice	2xCaMV35S, OsU3	*OsPDS, OsBADH2*	7.1% (9/97)–9.4% (7/98)	PCR + RE				Shan et al. (2013)
O. sativa	Agro-transformation of callus	Human	OsUBQ, OsU6	*OsMYB1*	50% (10/20)	Surveyor assay				Mao et al. (2013)
O. sativa	Agro-transformation of callus	Rice	ZmUbi, OsU3	*CAO1, LAZY1*	83.3% (25/30)–91.6%(11/12)	PCR + sequencing				Miao et al. (2013)
O. sativa	Agro-transformation of callus	Human	CaMV35S, OsU6	*ROCS, SPP, YSA*	5% (1/21)–75% (24/32)	PCR + RE				Feng et al. (2013)
O. sativa	Agro-transformation of callus	Rice	2xCaMV35S, ZmU3	*PDS, PMS3, EPSPS, DERF1, MSH1, MYB5, MYB1, ROCS, SPP, YSA*	21.1–66.7%	PCR + sequencing	No/whole-genome sequencing	Yes	Yes	Zhang et al.(2014)
O. sativa	Agro-transformation of callus	Plant	2xCaMV35S, AtU6	*BAL*	2% (2/94)–16% (14/90)	PCR + sequencing	No (3 sites)/PCR + sequencing			Xu et al. (2014)
O. sativa	Agro-transformation of callus	Human	2xCaMV35S, AtU6	*BAL*	0% (0/96)–2% (2/96)	PCR + sequencing				Xu et al. (2014)
O. sativa	Agro-transformation of callus	Rice	ZmUbi, OsU6	*SWEET1a-1b-11-13*	12.5% (2/16)–100% (8/8)	T7E1 assay + sequencing	No (6 sites)/PCR + sequencing		Yes	Zhou et al. (2014)
Sorghum bicolor	Agro-transformation of immature embryos	Monocot	OsActin1, OsU6	Co-transfected *DsRed*	28% (5/18)	DsRed fluorescence				Jia et al. (2014)
Solanum lycopersicum	Hairy root transformation by *A. rhizogenes*	*Nicotiana*	CaMV35S, AtU6	*GFP, SHR*	n.d.	RE + PCR; phenotype				Ron et al. (2014)
S. lycopersicum	Agro-transformation of cotyledons	Human	2xCaMV35S, AtU6	*SlAGO7, Solyc08g041770, Solyc07g021170, Solyc12g044760*	75 (6/8)–100% (29/29, 8/8)	phenotype, PCR + sequencing		Yes (90–140 bp)	Yes	Brooks et al. (2014)
N. tabacum	Agro-transformation of leaf discs	Tobacco	2xCaMV35S, AtU6	*PDS, PDR6*	82% (9/11)–88%(14/16)	Phenotype, PCR + RE sequencing	No (1 site)/PCR + RE		Yes	Gao et al. (2014)
T. aestivum	Particle bombardment of embryos	Plant	ZmUbi, TaU6	*MLO-A1*	5.6% (4/72)	T7E1			Yes	Brooks et al. (2014)
Marchantia polymorpha	Agro-transformation of sporelings	Human	CaMV35S or MpEF, MpU6	*ARF1*	11% (5/45)	Phenotype, PCR + sequencing				Sugano et al. (2014)

AtUBQ1, U6 = *A. thaliana* ubiquitin 1 or U6 promoter; PcUBI4-2 = *P. crispum* polyubiquitin promoter; CaMV35S = promoter of the 35S gene of the *cauliflower mosaic virus*; 2x35SCaMV = 35S promoter with duplicated enhancer; OsUBQ, Actin1, U3, U6 = *O. sativa* ubiquitin, actin 1, U3 or U6 promoters; ZmUbi, U3 = *Z. mays* ubiquitin or U3 promoters; TaU6 = *T. aestivum* U6 promoter; MpEF = *M. polymorpha* elongation factor 1α promoter; MpU6 = *M. polymorpha* U6 promoter; PCR = polymerase chain reaction; RE = restriction enzyme digestion; T7E1 = T7 endonuclease I assay; n.d. = not determined.

Molecular Plant
Review Article

CellPress
PARTNER JOURNAL

CRISPR/Cas9 Platforms for Genome Editing in Plants: Developments and Applications

Xingliang Ma[1,2,3], Qinlong Zhu[1,2,3], Yuanling Chen[1,2,3] and Yao-Guang Liu[1,2,3,*]

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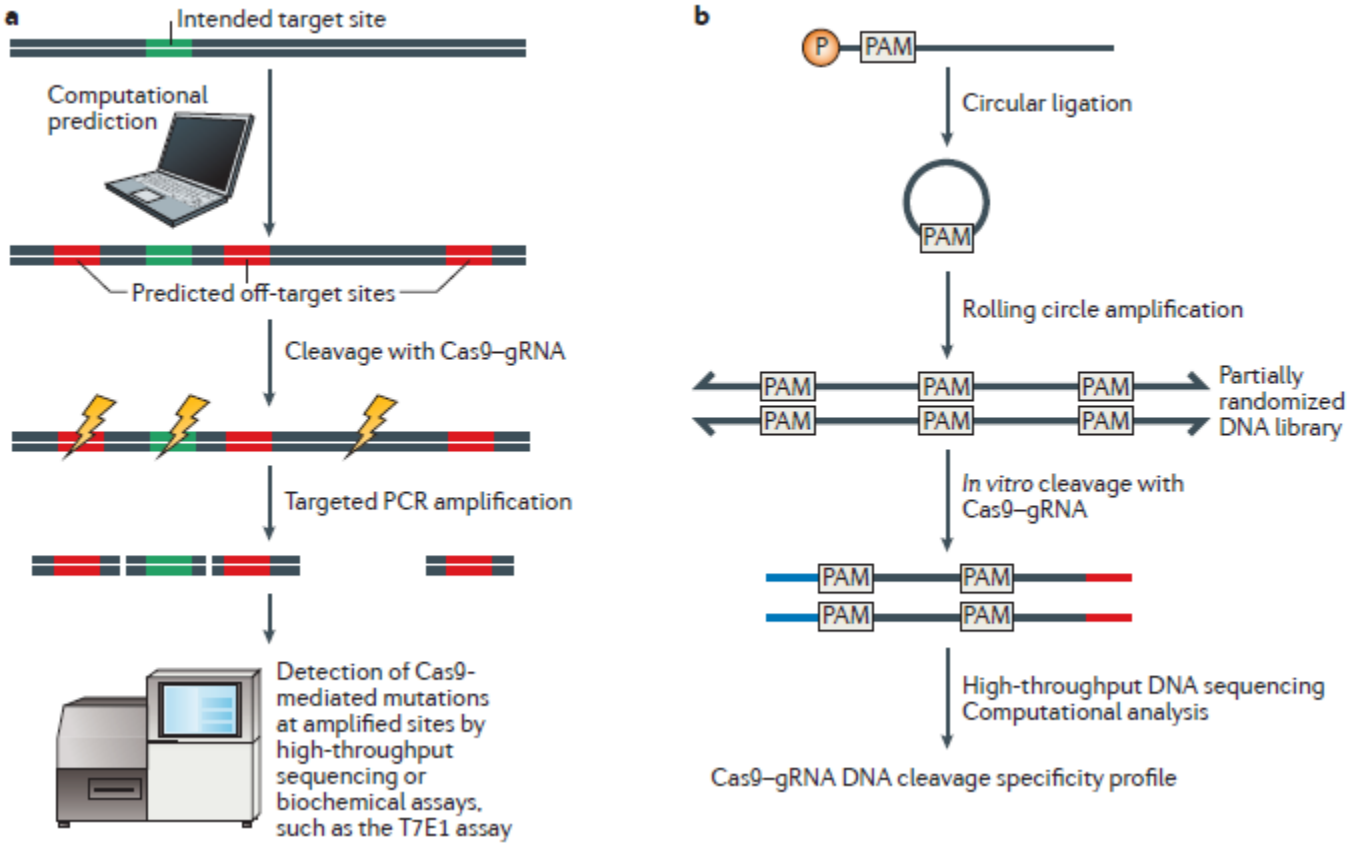
http://dx.doi.org/10.1016/j.molp.2016.04.009

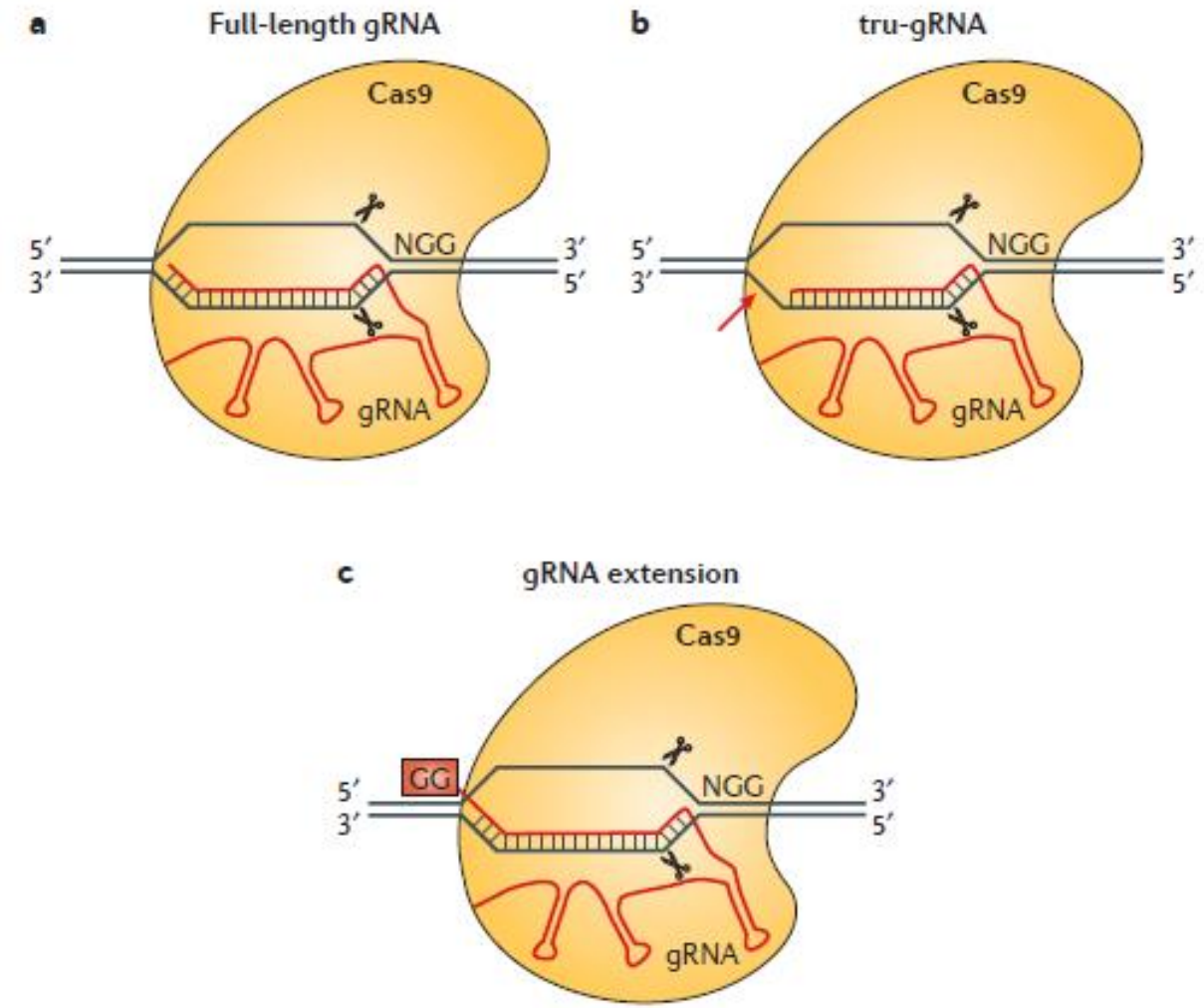
Plant species	Target gene	Function	Knockout/-in, phenotype	References
O. sativa	*CAO1*	Synthesis of chlorophyll *b*	Knockout, pale-green leaf	Miao et al., 2013
	LAZY1	Regulate shoot gravitropism, control tiller angle	Knockout, pronounced tiller spreading	Miao et al., 2013
	OsPDS	Encoding phytoene desaturase	Knockout, albino and dwarf	Shan et al., 2013
	Promoter region of *OsSWEET*	Plant disease susceptibility genes	Modification of the promoter, plant disease resistance	Zhou et al., 2014a
	OsWaxy	Amylose synthesis	Knockout, glutinous rice	Ma et al., 2015b
	ALS	Encoding acetolactate synthase involved in branched amino acid biosynthesis	Knockin, resistance to sulfonylurea herbicides, gene replacement	Endo et al., 2016
	ALS	Encoding acetolactate synthase involved in branched amino acid biosynthesis	Knockin, resistance to sulfonylurea herbicides	Sun et al., 2016
A. thaliana	*CHL1* *CHL2*	The magnesium chelatase subunit I genes	Knockout, albino (double mutant); pale green (either mutant)	Mao et al., 2013
	ABP1	Vital for perception of auxin and developmental processes	Knockout, no identifiable phenotype	Gao et al., 2015b
	ADH1	Turns allyl alcohol to highly toxic acrylaldehyde	Knockout, survival after allyl alcohol treatment	Fauser et al., 2014
	NPTII	Kanamycin resistance cassette	Knockin, kanamycin resistance	Schiml et al., 2014
	Beet severe curly top virus	Virus infection	Suppression, virus resistance	Ji et al., 2015
	TRL1	–	Knockin, eGFP expression	Zhao et al., 2016
N. benthamiana	*NbPds*	Phytoene desaturase	Knockout, albino	Gao et al., 2015a
	NbPDR6	One ABC transporter participating in strigolactone transport	Knockout, more branches	Gao et al., 2015a
	Bean yellow dwarf virus (BeYDV)	Virus infection	Suppression, virus resistance	Baltes et al., 2015
	Tomato yellow leaf curl virus (TYLCV); Beet curly top virus (BCTV); Merremia mosaic virus (MeMV)	Virus infection	Suppression, virus resistance (transient expression)	Ali et al., 2015b
S. lycopersicum	*SlAGO7*	Biogenesis of *trans*-acting short interfering RNAs	Knockout, needle-like or wiry leaves	Brooks et al., 2014
T. aestivum	*TaMLO* homologs	Repress the resistance pathway to powdery mildew	Knockout, resistance to powdery mildew	Wang et al., 2014
H. vulgare	*HvPM19*	ABA-inducible plasma membrane protein	Knockout, related to grain dormancy	Lawrenson et al., 2015
B. oleracea	*BoIC.GA4.*	Ortholog of *Arabidopsis GA4a*	Knockout, dwarf phenotype	Lawrenson et al., 2015

Defining and improving the genome-wide specificities of CRISPR–Cas9 nucleases

Shengdar Q. Tsai and J. Keith Joung



Evitando *off-targets*…



Evitando *off-targets*…

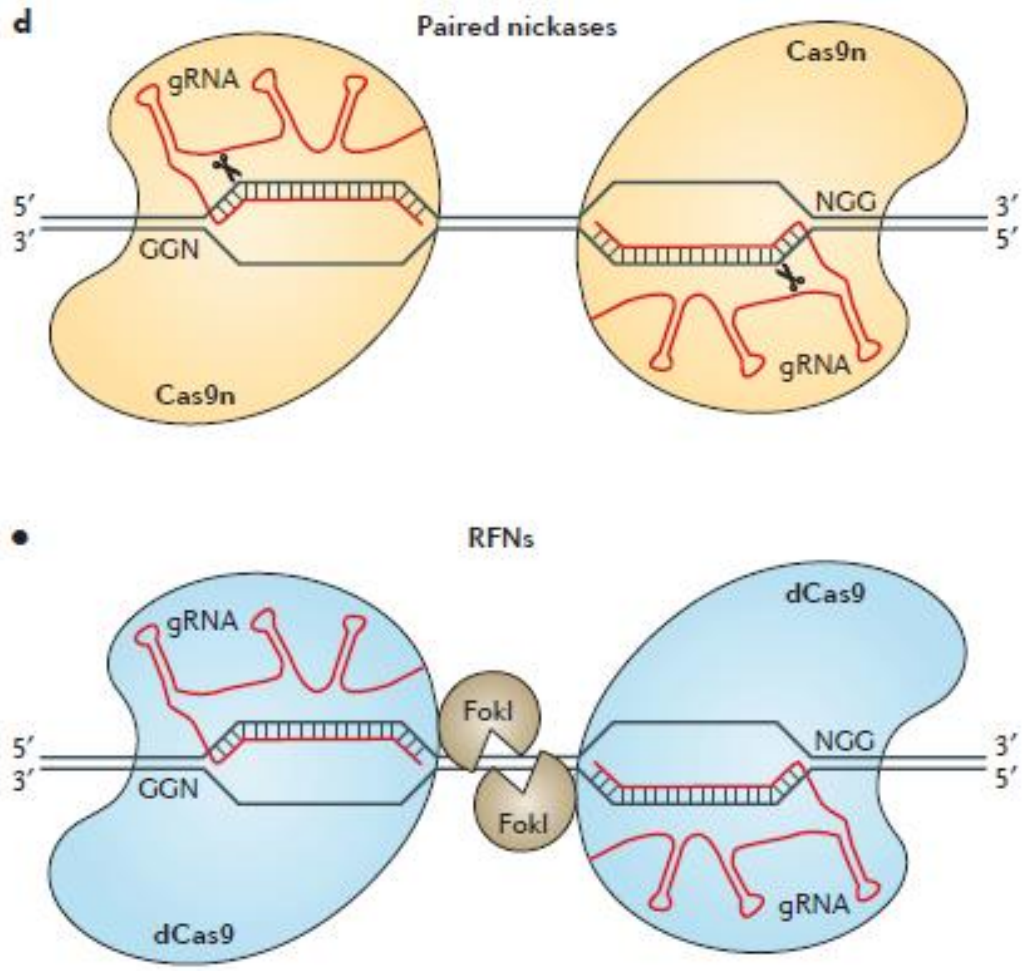
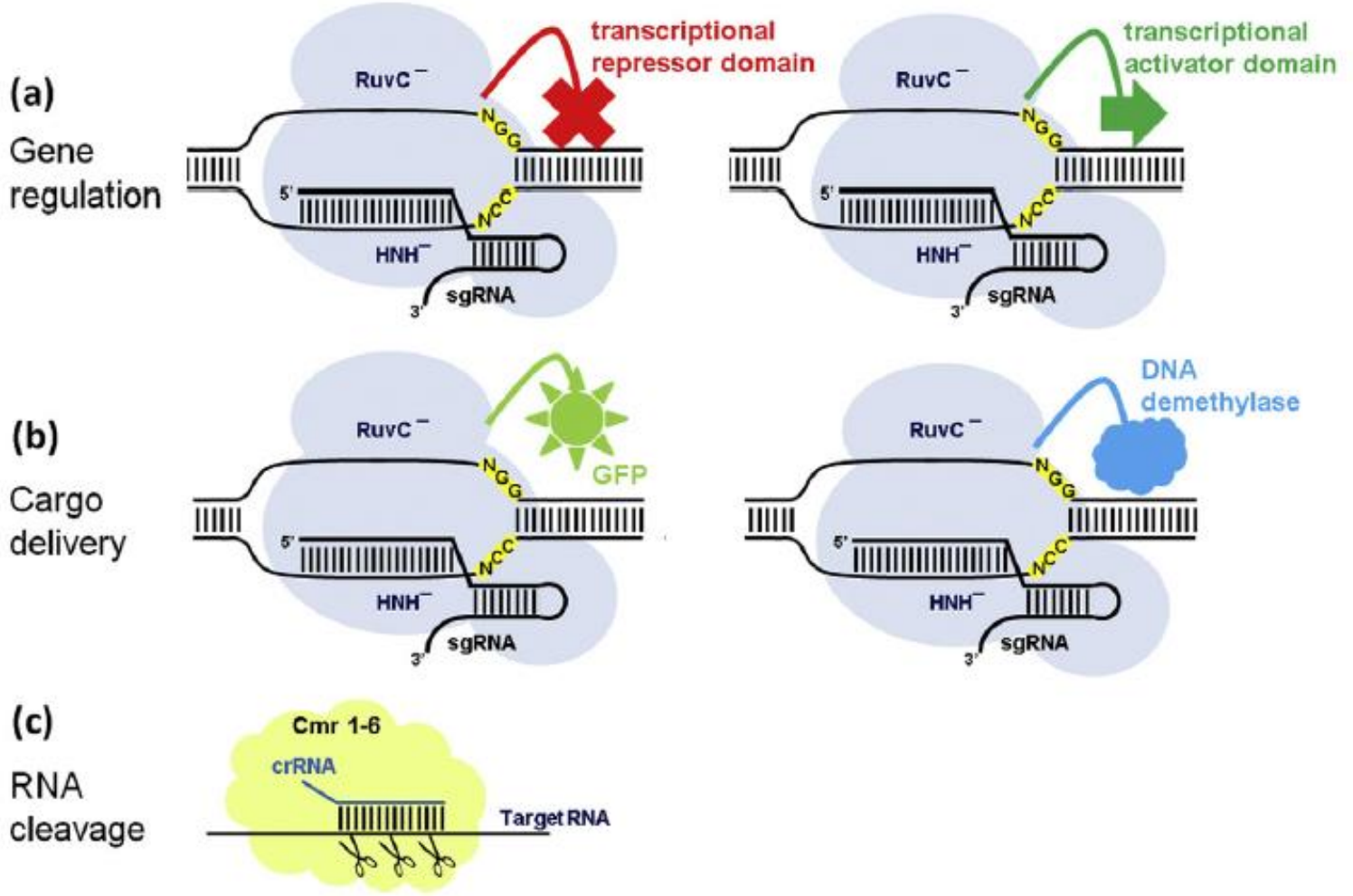


Table 1 | **Comparison of strategies for improving CRISPR–Cas9 specificity**

Strategy	Description	Mechanism of action	Specificity characterization
tru-gRNAs[60] (FIG. 3b)	gRNA truncated by 2–3 nucleotides at the 5′ end	May reduce excess interaction energy at RNA–DNA interface	Targeted high-throughput sequencing and GUIDE-seq; number of genome-wide off-target sites is reduced; most off-target sites detected have two or fewer mismatches compared to intended target site[53]
Extended gRNAs[32] (FIG. 3c)	Two G nucleotides added to the 5′ end of the gRNA	The mechanism of increased specificity is unclear, but may involve destabilization of protein interactions with the 5′ end of the gRNA	Targeted high-throughput sequencing; reduction in off-target effects observed with certain gRNAs; in some cases, on-target activity is also reduced[32,58]
Cas9n[61,62] (FIG. 3d)	Co-localization of paired Cas9n in which one of the nuclease domains has been catalytically inactivated	The requirement for two proximal single-strand breaks (nicks) on opposite DNA strands of the target site (guided by distinct gRNAs) is thought to limit the propensity for off-target nicks of either gRNA–nickase to result in DSBs	Targeted sequencing and HTGTS; number of off-target sites detected is generally reduced; monomeric activity is low but certain sites retain high mutagenesis frequencies[32,60,63,61]
RFNs[63,64] (FIG. 3e)	Fusion of catalytically inactive dCas9 to dimerization-dependent non-specific FokI nuclease	Similar to paired nickases, generation of DSBs typically requires binding of a pair of separately targeted monomers on opposite DNA strands at the target site but, unlike paired nickases, unpaired monomers are expected to be catalytically inactive because FokI dimerization is required for activity	Targeted high-throughput sequencing of known monomeric and predicted dimeric sites; background or near-background off-target activity detected by high-throughput sequencing; has not yet been fully characterized by genome-wide methods[63,64,65]
Engineered Cas9 variants[68,69] (FIG. 3f)	Introduce alanine substitutions at 3–4 residues in SpCas9	Reduce Cas9 non-specific DNA interactions with target (SpCas9-HF1) or non-target strand (eSpCas9 1.1)	Targeted high-throughput sequencing, GUIDE-seq or BLESS; number of detectable genome-wide off-target sites is reduced or eliminated; at certain off-target sites high-frequency mutagenesis remains possible[68,69]

HÁ UM UNIVERS0 PARA SER EXPLORADO…



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!!!!

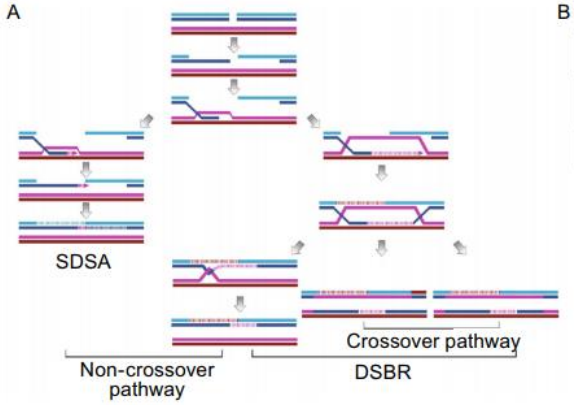
Protein Cell
DOI 10.1007/s13238-015-0153-5

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✉



B

3PN zygotes edited through non-crossover HR	
HBB-HBD	Non-crossover
7	5*

LETTER

doi:10.1038/nature17664

Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9

Dominik Paquet[1]*, Dylan Kwart[1]*, Antonia Chen[1], Andrew Sproul[2]†, Samson Jacob[2], Shaun Teo[1], Kimberly Moore Olsen[1], Andrew Gregg[1,3], Scott Noggle[2] & Marc Tessier-Lavigne[1]

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

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]

nature biotechnology

High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells

Yanfang Fu[1–4], Jennifer A Foden[1–3], Cyd Khayter[1–3], Morgan L Maeder[1–3,5], Deepak Reyon[1–4], J Keith Joung[1–5] & Jeffry D Sander[1–4]

Table 2 Indel mutation frequencies at on- and off-target genomic sites induced by different amounts of Cas9- and sgRNA-expressing plasmids for the RGN targeted to *VEGFA* target site 2

		250 ng sgRNA/ 750 ng Cas9	12.5 ng sgRNA/ 50 ng Cas9
Site name	Sequence	Mean indel frequency (%) ± s.e.m.	Mean indel frequency (%) ± s.e.m.
T2 (on-target)	GACCCCCTCCACCCCGCCTCCGG	50.2 ± 4.9	25.4 ± 4.8
OT2-1	GACCCCCCCCACCCCGCCCCCGG	14.4 ± 3.4	4.2 ± 0.2
OT2-2	GGGCCCCTCCACCCCGCCTCTGG	20.0 ± 6.2	9.8 ± 1.1
OT2-6	CTACCCCTCCACCCCGCCTCCGG	8.2 ± 1.4	6.0 ± 0.5
OT2-9	GCCCCCACCCACCCCGCCTCTGG	50.7 ± 5.6	16.4 ± 2.1
OT2-15	TACCCCCCACACCCCGCCTCTGG	9.7 ± 4.5	2.1 ± 0.0
OT2-17	ACACCCCCCCACCCCGCCTCAGG	14.0 ± 2.8	7.1 ± 0.0
OT2-19	ATTCCCCCCCACCCCGCCTCAGG	17.0 ± 3.3	9.2 ± 0.4
OT2-20	CCCCACCCCCACCCCGCCTCAGG	6.1 ± 1.3	N.D.
OT2-23	CGCCCTCCCCACCCCGCCTCCGG	44.4 ± 6.7	35.1 ± 1.8
OT2-24	CTCCCCACCCACCCCGCCTCAGG	62.8 ± 5.0	44.1 ± 4.5
OT2-29	TGCCCCTCCCACCCCGCCTCTGG	13.8 ± 5.2	5.0 ± 0.2
OT2-34	AGGCCCCCACACCCCGCCTCAGG	2.8 ± 1.5	N.D.

Amounts of sgRNA- and Cas9-expressing plasmids transfected into U2OS.EGFP cells for these assays are shown at the top of each column. (Note that data for 250 ng sgRNA/750 ng Cas9 are the same as those presented in **Table 1**.) Mean indel frequencies were determined using the T7EI assay from replicate samples, as described in Online Methods. OT, off-target sites, numbered as in **Table 1** and **Supplementary Table 2**. Mismatches from the on-target site (within the 20 bp region to which the gRNA hybridizes) are boldface and underlined. N.D., none detected.

doi:10.1038/nbt.2623

Table 1 On- and off-target mutations induced by RGNs designed to endogenous human genes

Target	Site name	Sequence	Indel mutation frequency (%) ± s.e.m. U2OS.EGFP	HEK293	K562	Gene
Target 1 (*VEGFA* site 1)	T1	GGGTGGGGGGAGTTTGCTCCTGG	26.0 ± 2.9	10.5 ± 0.07	3.33 ± 0.42	*VEGFA*
	OT1-3	GG**A**TGG**A**GGGAGTTTGCTCCTGG	25.7 ± 9.1	18.9 ± 0.77	2.93 ± 0.04	*IGDCC3*
	OT1-4	GGG**A**GGG**T**GGAGTTTGCTCCTGG	9.2 ± 0.8	8.32 ± 0.51	N.D.	*LOC116437*
	OT1-6	**C**GGG**G**GG**A**GGGAGTTTGCTCCTGG	5.3 ± 0.2	3.67 ± 0.09	N.D.	*CACNA2D*
	OT1-11	GGG**GA**GGGG**A**AGTTTGCTCCTGG	17.1 ± 4.7	8.54 ± 0.16	N.D.	
Target 2 (*VEGFA* site 2)	T2	GACCCCCTCCACCCCGCCTCCGG	50.2 ± 4.9	38.6 ± 1.92	15.0 ± 0.25	*VEGFA*
	OT2-1	GACCCCC**C**CCACCCCGCC**C**CCGG	14.4 ± 3.4	33.6 ± 1.17	4.10 ± 0.05	*FMN1*
	OT2-2	G**GG**CCCCTCCACCCCGCCTCTGG	20.0 ± 6.2	15.6 ± 0.30	3.00 ± 0.06	*PAX6*
	OT2-6	**CTA**CCCCTCCACCCCGCCTCCGG	8.2 ± 1.4	15.0 ± 0.64	5.24 ± 0.22	*PAPD7*
	OT2-9	G**C**CCCC**AC**CCACCCCGCCTCTGG	50.7 ± 5.6	30.7 ± 1.44	7.05 ± 0.48	*LAMA3*
	OT2-15	**T**ACCCCC**CA**CACCCCGCCTCTGG	9.7 ± 4.5	6.97 ± 0.10	1.34 ± 0.15	*SPNS3*
	OT2-17	**ACA**CCCC**C**CCACCCCGCCTCAGG	14.0 ± 2.8	12.3 ± 0.45	1.80 ± 0.03	
	OT2-19	**ATT**CCCC**C**CCACCCCGCCTCAGG	17.0 ± 3.3	19.4 ± 1.35	N.D.	*HDLBP*
	OT2-20	**CC**CC**A**CC**C**CCACCCCGCCTCAGG	6.1 ± 1.3	N.D.	N.D.	*ABLIM1*
	OT2-23	**CG**CCC**T**C**C**CCACCCCGCCTCCGG	44.4 ± 6.7	28.7 ± 1.15	4.18 ± 0.37	*CALY*
	OT2-24	**CT**CCCC**AC**CCACCCCGCCTCAGG	62.8 ± 5.0	29.8 ± 1.08	21.1 ± 1.68	
	OT2-29	**TG**CCCC**TC**CCACCCCGCCTCTGG	13.8 ± 5.2	N.D.	N.D.	*ACLY*
	OT2-34	**AGG**CCCC**CA**CACCCCGCCTCAGG	2.8 ± 1.5	N.D.	N.D.	
Target 3 (*VEGFA* site 3)	T3	GGTGAGTGAGTGTGTGCGTGTGG	49.4 ± 3.8	35.7 ± 1.26	27.9 ± 0.52	*VEGFA*
	OT3-1	GGTGAGTGAGTGTGTG**T**GTGAGG	7.4 ± 3.4	8.97 ± 0.80	N.D.	*(abParts)*
	OT3-2	**A**GTGAGTGAGTGTGTG**T**GTGGGG	24.3 ± 9.2	23.9 ± 0.08	8.9 ± 0.16	*MAX*
	OT3-4	G**C**TGAGTGAGTGT**A**TGCGTGTGG	20.9 ± 11.8	11.2 ± 0.23	N.D.	
	OT3-9	GGTGAGTGAGTG**C**GTGCG**G**GTGG	3.2 ± 0.3	2.34 ± 0.21	N.D.	*TPCN2*
	OT3-17	G**T**TGAGTGA**A**TGTGTGCGTGAGG	2.9 ± 0.2	1.27 ± 0.02	N.D.	*SLIT1*
	OT3-18	**T**GTG**G**GTGAGTGTGTGCGTGAGG	13.4 ± 4.2	12.1 ± 0.24	2.42 ± 0.07	*COMDA*
	OT3-20	**A**G**A**GAGTGAGTGTGTGC**A**TGAGG	16.7 ± 3.5	7.64 ± 0.05	1.18 ± 0.01	
Target 4 (*EMX1*)	T4	GAGTCCGAGCAGAAGAAGAAGGG	42.1 ± 0.4	26.0 ± 0.70	10.7 ± 0.50	*EMX1*
	OT4-1	GAGT**TA**GAGCAGAAGAAGAAAGG	16.8 ± 0.2	8.43 ± 1.32	2.54 ± 0.02	*HCN1*
Target 5 (*RNF2*)	T5	GTCATCTTAGTCATTACCTGTGG	26.6 ± 6.0	—	—	*RNF2*
Target 6 (*FANCF*)	T6	GGAATCCCTTCTGCAGCACCAGG	33.2 ± 6.5	—	—	*FANCF*

OT indicates off-target sites (with numbering of sites as in **Supplementary Table 2**). Mismatches from the on-target (within the 20 bp region to which the sgRNA hybridizes) are boldface and underlined. Mean indel mutation frequencies in U2OS.EGFP, HEK293 and K562 cells were determined as described in Online Methods. Genes in which sites were located (if any) are shown. All sites listed failed to show any evidence of modification in cells transfected with Cas9 expression plasmid and a control U6 promoter plasmid that did not express a functional sgRNA. N.D., none detected; —, not tested.

doi:10.1038/nbt.2623

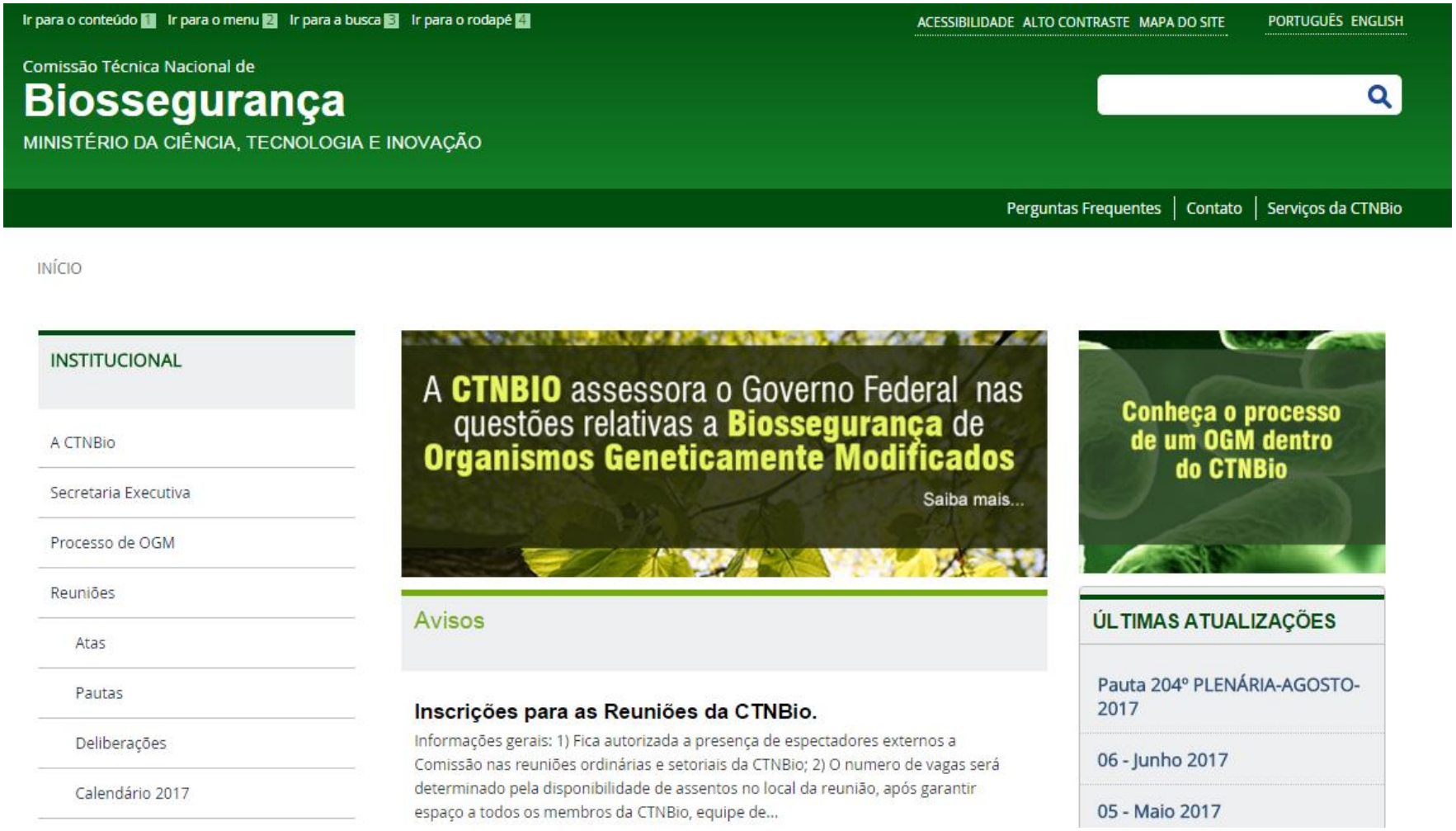
QUEM CONTROLA A LIBERAÇÃO DE ORGANISMOS GENETICAMENTE MODIFICADOS?

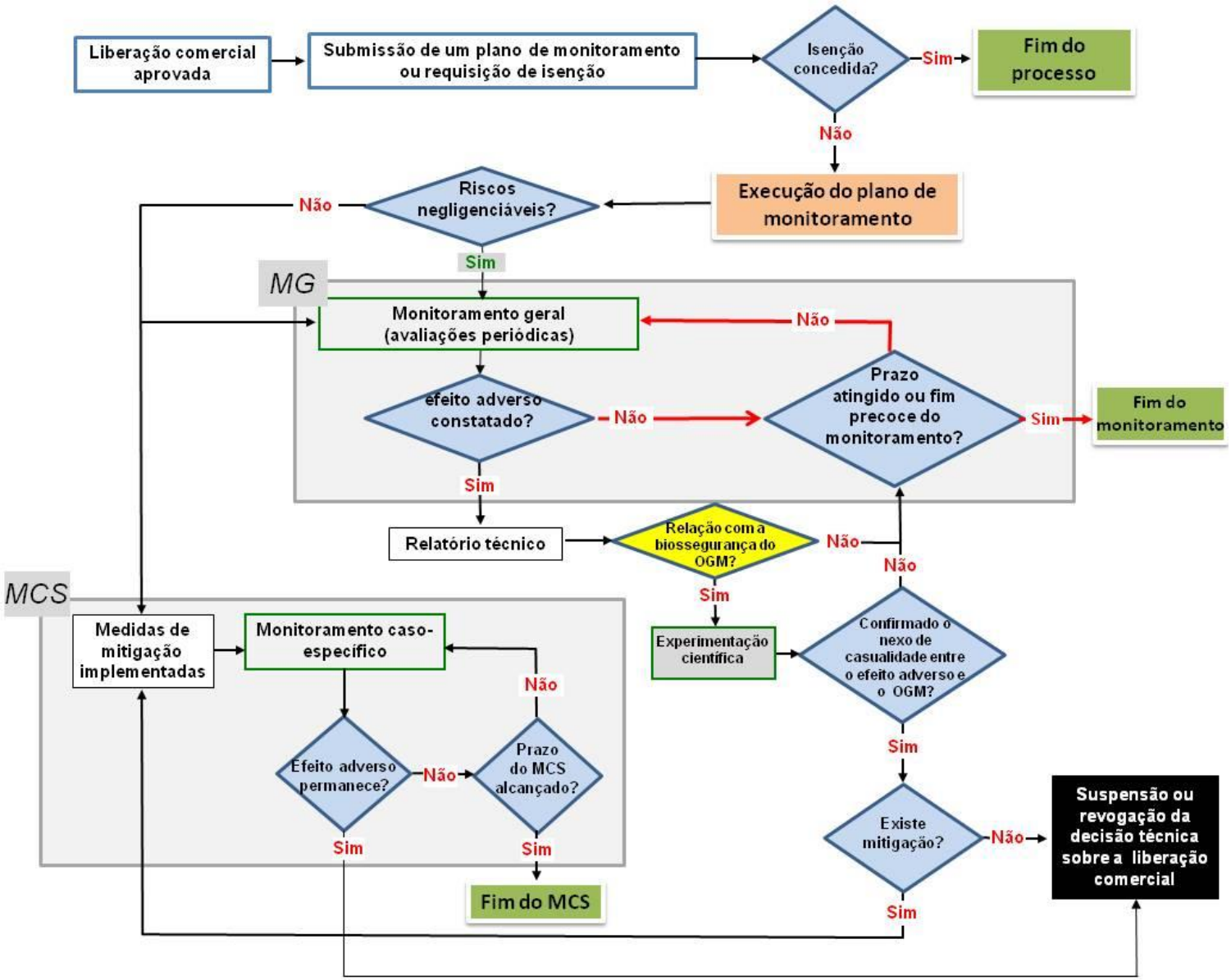
TODO ORGANISMO GENETICAMENTE MODIFICADO É UM TRANSGÊNICO?

TRANSGÊNICOS
Geneticamente Modificados

ELES SÃO SEGUROS?

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

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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 »
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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 e CRISPR??

OGM ou não?

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/

http://www.abbi.org.br/pt/noticia/decisao-da-ctnbio-sobre-tecnicas-inovadoras-de-melhoramento-de-precisao-e-um-marco-para-biotecnologia-industrial-brasileira/

8 DE JUNHO DE 2018

Decisão da CTNBio sobre organismo produzido por Técnica Inovadora de Melhoramento de Precisão é um marco para a biotecnologia industrial brasileira

CRISPR permite acelerar o processo de produção de OGMs

http://plantproject.com.br/novo/2019/02/agribusiness-13-crispr-a-sigla-do-futuro-do-agro/

Reportagem recente...

FOMENTO

Aprendizado em edição de genoma

Instituto Agronômico vai utilizar "tesoura genética" para solucionar problemas nas culturas de cana, café e citros

Fabrício Marques

Edição 305
jul. 2021

Atualizado em
7 jul 2021

Agronomia
Genética

Uma rede de pesquisadores sediada no Instituto Agronômico (IAC) vai utilizar técnicas de edição de genoma para buscar ampliar a qualidade e a produtividade de três culturas agrícolas de grande importância econômica: o café, a cana-de-açúcar e os citros. O esforço de pesquisa tem focos específicos, como o desenvolvimento de café com baixíssimo teor de cafeína, de laranjais resistentes a uma doença conhecida como HLB ou greening e de variedades de cana aprimoradas. Esses alvos são um pretexto para alcançar um objetivo mais ambicioso. A intenção é criar na comunidade de pesquisa em agronomia do Brasil uma expertise no uso da ferramenta CRISPR-Cas9, sigla de repetições palindrômicas curtas agrupadas e regularmente interespaçadas (*ver* Pesquisa FAPESP *nº 288*). O método funciona como uma espécie de tesoura e permite remover ou adicionar com precisão trechos do DNA de seres humanos, animais, plantas ou microrganismos. Seu potencial para criar tratamentos de doenças genéticas foi reconhecido com o Prêmio Nobel de Química de 2020, concedido às pesquisadoras Emmanuelle Charpentier, francesa, e Jennifer Doudna, norte-americana.

https://revistapesquisa.fapesp.br/aprendizado-em-edicao-de-genoma/

14/12/21 | AGROENERGIA MELHORAMENTO GENÉTICO PESQUISA, DESENVOLVIMENTO E INOVAÇÃO PRODUÇÃO VEGETAL

Ciência brasileira desenvolve primeira cana editada não-transgênica do mundo

Foto: Hugo Molinari

Assunto: Consulta Prévia - Resolução Normativa 16

Decisão: Não OGM

A CTNBio, após análise de Requerimento de Consulta Prévia a respeito do Enquadramento Regulatório do Produto Pivot Bio Kv137-2253 (Klebsiella variicola cepa 137-2253) obtido por Técnicas Inovadoras de Melhoramento de Precisão (TIMP) nos termos da Lei nº 11.105 de 24 de março de 2005 e da Resolução Normativa Nº 16, de 15 de janeiro de 2018, concluiu que não se trata de um organismo geneticamente modificado à luz da legislação brasileira. Trata-se 01245.014231/2022-12 de um inoculante em pó para uso no tratamento de sementes de milho antes do plantio, contendo a bactéria diazotrófica Klebsiella variicola cepa 137- 2253 que foi geneticamente editada por meio da tecnologia de plasmídeo suicida para aumentar sua capacidade de produzir amônia, um importante nutriente absorvido pela raiz da planta para sustentar o seu crescimento. Isso é alcançado por meio da alteração da via de fixação de nitrogênio (operon nif) localizada na região conservada do genoma de Klebsiella variicola, resultando no aumento da taxa de fixação de nitrogênio e produção de amônia, e, consequentemente, maior produtividade da cultura do milho com menor consumo de fertilizantes químicos nitrogenados. O produto implica nas características listadas: Produto com ausência comprovada de ADN/ARN recombinante, obtido por técnica que emprega OGM como parental; 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; 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, visto que não existem moléculas de DNA/RNA recombinantes no produto Kv 137-1034.

PRÓXIMA AULA – 28 de Junho

Aula – Biologia Sintética – Profa. Dra. Elizabeth Bilsland