

Clustered

Regularly

Interspaced

Short

Palindromic

Repeats



EVERYWHERE

Svantaggi dell'ingegneria genetica

L'inserzione del transgene nel genoma nucleare avviene mediante ricombinazione illegittima

Impossibilità di prevedere il sito di inserzione del transgene

Diversi livelli di trascrizione a seconda della regione del genoma in cui il transgene si è inserito

Necessità di ottenere numerose piante GM per selezionare quella con i livelli di trascrizione desiderati



Giappone

La prima osservazione di cluster di ripetizioni di DNA separate da DNA «spaziatore» avvenne all'università di Osaka (Giappone)

1987



Yoshizumi Ishino

JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429-5433
0021-9193/87/125429-05\$02.00/0
Copyright © 1987, American Society for Microbiology

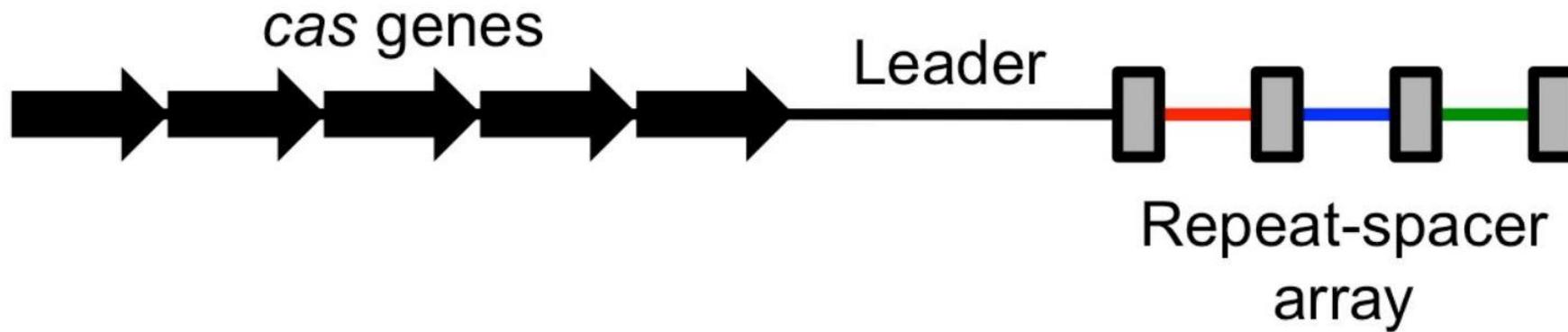
Vol. 169, No. 12

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

YOSHIZUMI ISHINO, HIDEO SHINAGAWA, KOZO MAKINO, MITSUKO AMEMURA, AND ATSUO NAKATA*

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

Received 1 May 1987/Accepted 22 August 1987



Variable Nucleotide Repeat Sequences

Individual 1

GGT AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG CGGTAAGTAGCGA

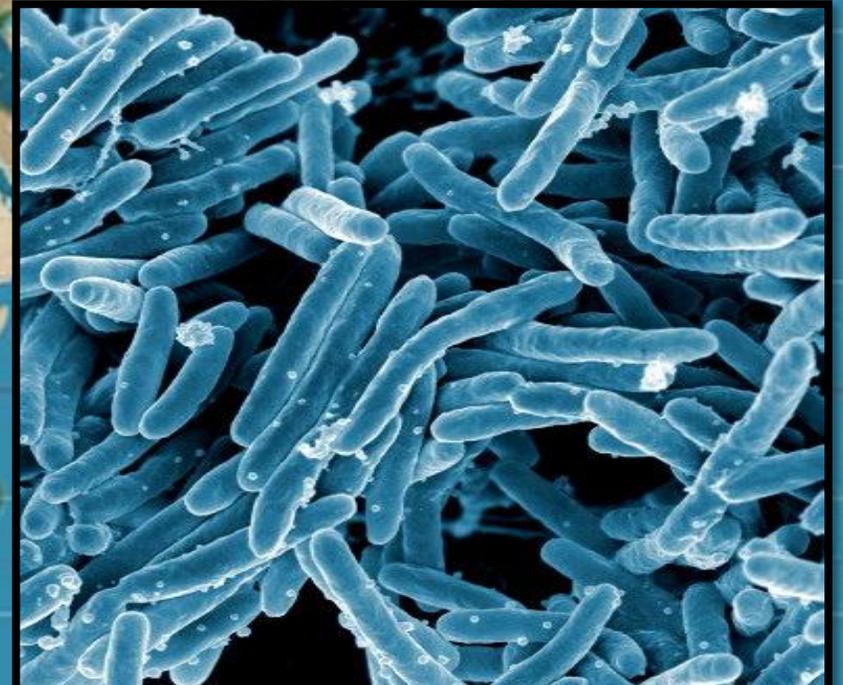
Individual 2

GGT AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG CGTT

Paesi Bassi

Durante lo studio del genoma di *Mycobacterium tuberculosis*, si osservarono delle sequenze interrotte di sequenze ripetute di DNA

1993



Mycobacterium tuberculosis



Spagna

Francisco Mojica scoprì la presenza di cluster di ripetizioni di DNA in alcune specie di archei del genere *Haloferax* e *Haloarcula*



Francisco Mojica

**molecular
microbiology**

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

F. J. M. Mojica, G. Juez, F. Rodriguez-Valera

First published: August 1993 | <https://doi.org/10.1111/j.1365-2958.1993.tb01721.x> | Citations: 95



Spagna

Francisco Mojica identificò ripetizioni interrotte in altre 20 specie di procarioti e ne descrisse la struttura



Francisco Mojica

molecular
microbiology

Free Access

Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria

Francisco J. M. Mojica ✉, Cesar Díez-Villaseñor, Elena Soria, Guadalupe Juez

First published: 18 January 2002 | <https://doi.org/10.1046/j.1365-2958.2000.01838.x> | Citations: 341

Germania

I loci CRISPR vengono trascritti

2002

RESEARCH ARTICLE



Identification of 86 candidates for small non-messenger RNAs from the archaeon *Archaeoglobus fulgidus*

Thean-Hock Tang, Jean-Pierre Bachellerie, Timofey Rozhdestvensky, Marie-Line Bortolin, Harald Huber, Mario Drungowski, Thorsten Elge, Jürgen Brosius, and Alexander Hüttenhofer

Paesi Bassi e USA

origine del termine CRISPR e geni del
DNA repair system

2002

Molecular Microbiology (2002) 43(6), 1565–1575

Identification of genes that are associated with DNA repeats in prokaryotes

Ruud. Jansen,^{1*} Jan. D. A. van Embden,²
Wim. Gastra¹ and Leo. M. Schouls²

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

Kira S. Makarova, L. Aravind, Nick V. Grishin, Igor B. Rogozin, Eugene V. Koonin

Nucleic Acids Research, Volume 30, Issue 2, 15 January 2002, Pages 482–496,

<https://doi.org/10.1093/nar/30.2.482>

Published: 15 January 2002

Francia e Spagna

Le sequenze spacer hanno origine fagica

2005

Published: February 2005

Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements

[Francisco J.M. Mojica](#) , [Chc\)sar Díez-Villaseñor](#), [Jesús García-Martínez](#) & [Elena Soria](#)

Journal of Molecular Evolution **60**, 174–182(2005) | [Cite this article](#)

29k Accesses | 977 Citations | 228 Altmetric | [Metrics](#)



MICROBIOLOGY Volume 151, Issue 8

Other | Free

Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin

Alexander Bolotin¹, Benoit Quinquis¹, Alexei Sorokin¹, S. Dusko Ehrlich¹

 [View Affiliations](#)

First Published: 01 August 2005 | <https://doi.org/10.1099/mic.0.28048-0>

MICROBIOLOGY Volume 151, Issue 3

Other | Free

CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies

C. Pourcel¹, G. Salvignol¹, G. Vergnaud^{1,2}

 [View Affiliations](#)

First Published: 01 March 2005 | <https://doi.org/10.1099/mic.0.27437-0>

USA e Francia

Il sistema CRISPR-Cas conferisce ai procarioti una resistenza acquisita contro i virus

2006

2007

Research | [Open Access](#) | Published: 16 March 2006

A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action

[Kira S. Makarova](#), [Nick V. Grishin](#), [Svetlana A. Shabalina](#), [Yuri I. Wolf](#) & [Eugene V. Koonin](#) 

[Biology Direct](#) **1**, Article number: 7 (2006) | [Cite this article](#)

59k Accesses | 663 Citations | 120 Altmetric | [Metrics](#)

REPORT

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, [Denni...](#)

+ See all authors and affiliations

Science 23 Mar 2007:
Vol. 315, Issue 5819, pp. 1709-1712
DOI: 10.1126/science.1138140

USA e Francia

Il sistema CRISPR-Cas conferisce ai procarioti una resistenza acquisita contro i virus

2006

2007



Streptococcus thermophilus

Research | [Open Access](#) | Published: 16 March 2006

A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action

[Kira S. Makarova](#), [Nick V. Grishin](#), [Svetlana A. Shabalina](#), [Yuri I. Wolf](#) & [Eugene V. Koonin](#) 

[Biology Direct](#) **1**, Article number: 7 (2006) | [Cite this article](#)

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REPORT

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

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+ See all authors and affiliations

Science 23 Mar 2007:
Vol. 315, Issue 5819, pp. 1709-1712
DOI: 10.1126/science.1138140

CRISPR

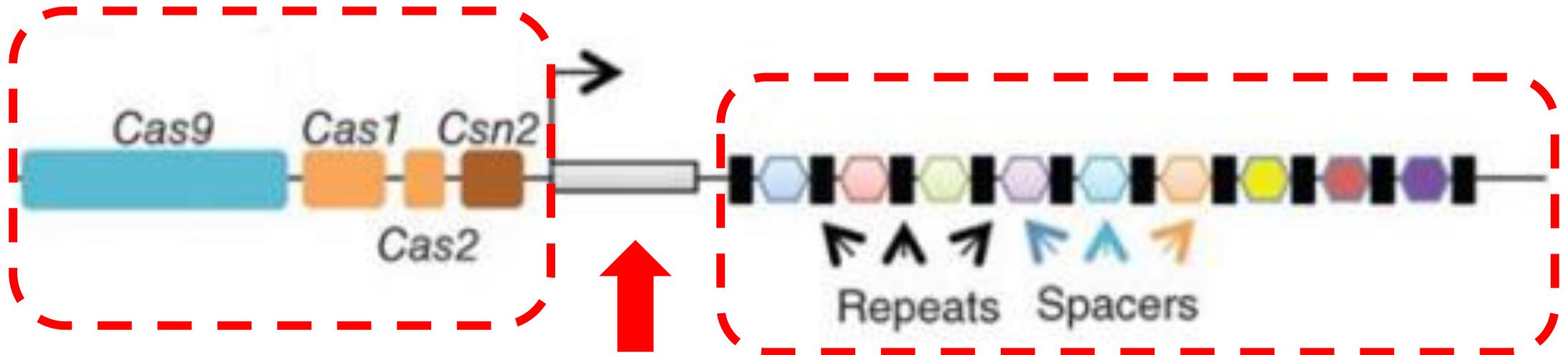
In natura CRISPR è un locus genico che è presente sia nei Batteri che negli Archea , il suo acronimo sta per *brevi ripetizioni palindrome raggruppate e separate a intervalli regolari.*

Per questi organismi rappresenta una forma di **immunità acquisita**, queste brevi ripetizioni infatti vengono sfruttate dal batterio per riconoscere e distruggere il genoma dei batteriofagi

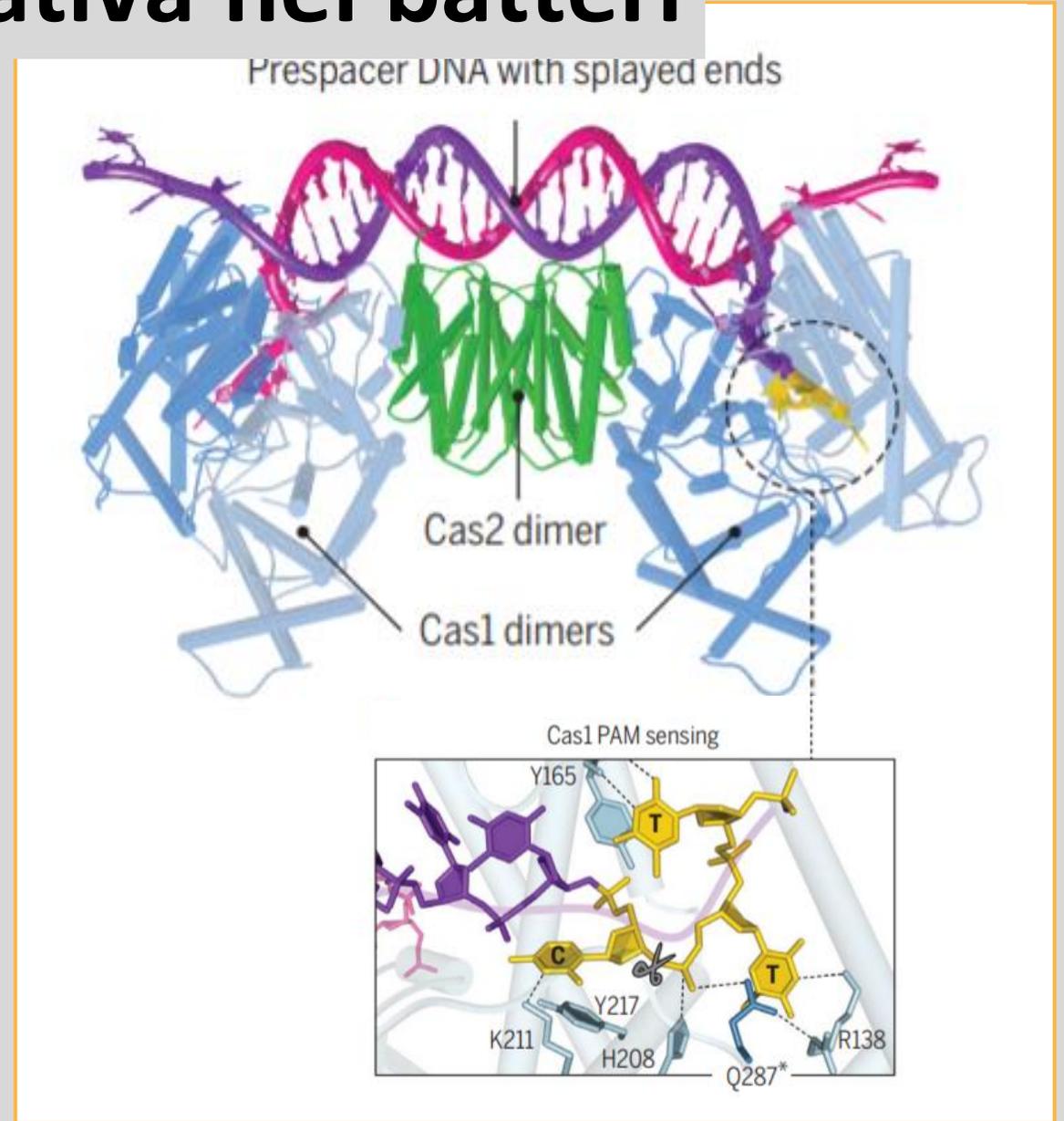
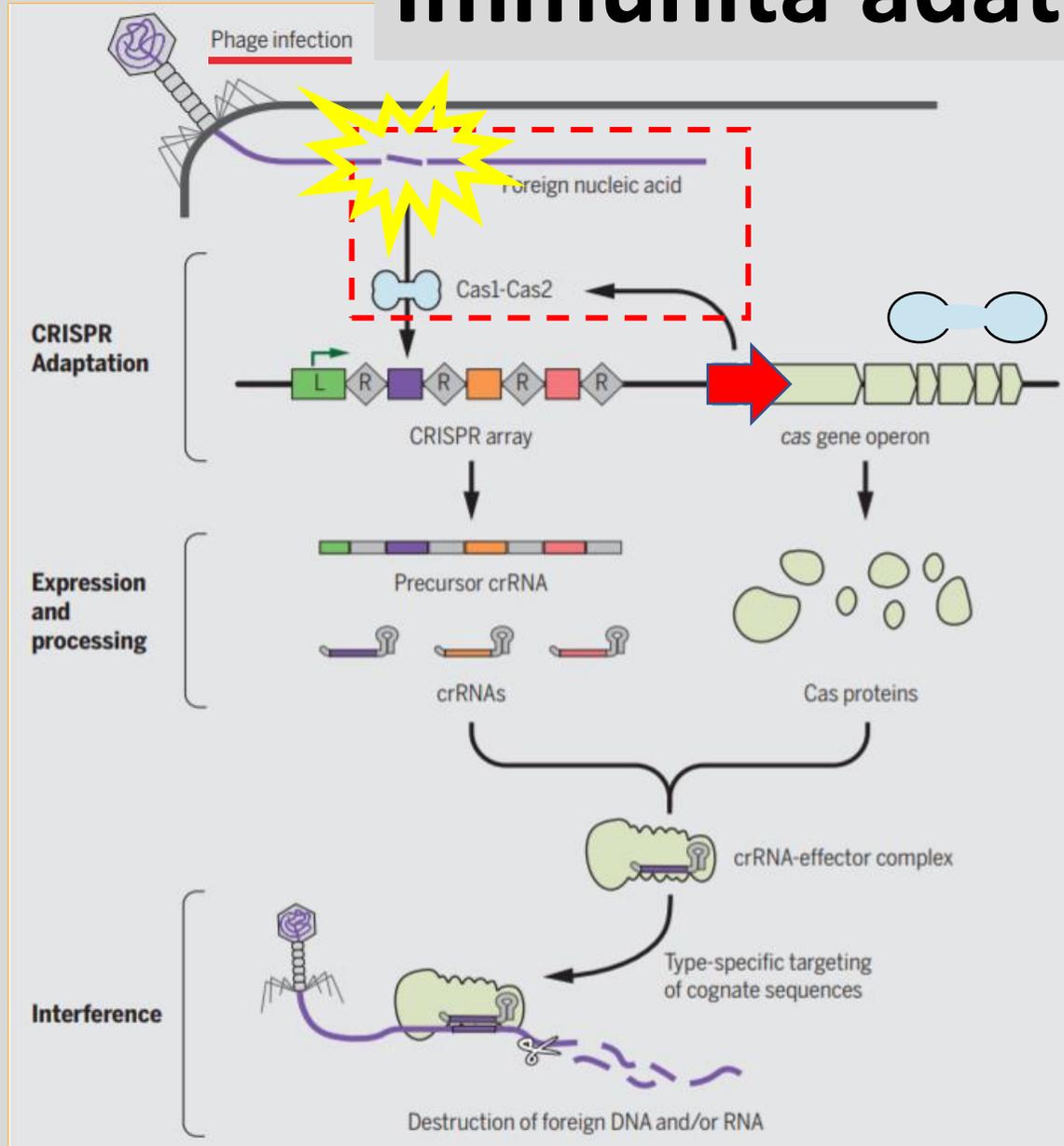


Il locus CRISPR è accompagnato da cluster definiti **CRISPR associated system**, da qui nasce l'acronimo, Cas, con il quale vengono chiamate le proteine associate a questo sistema.

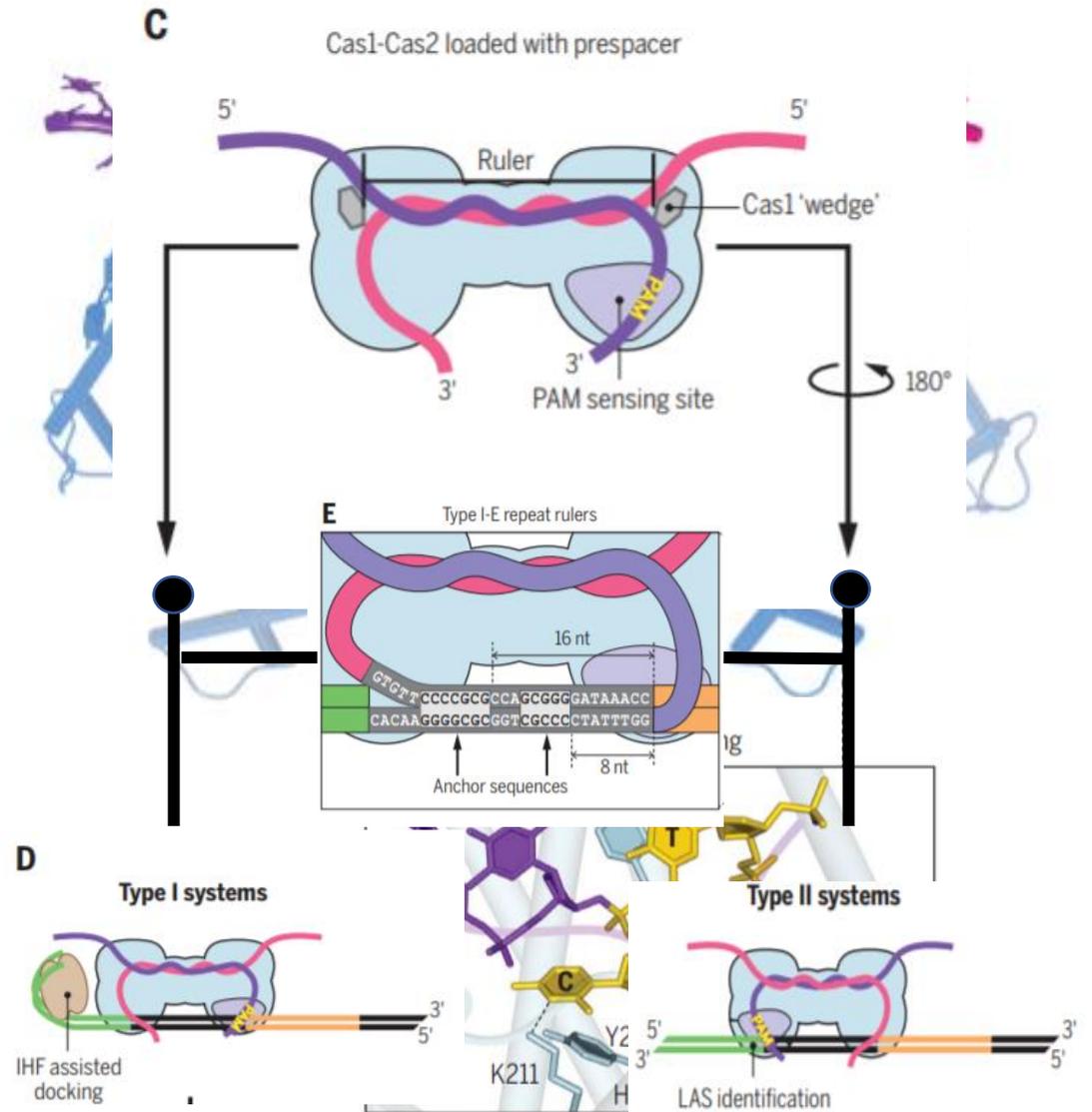
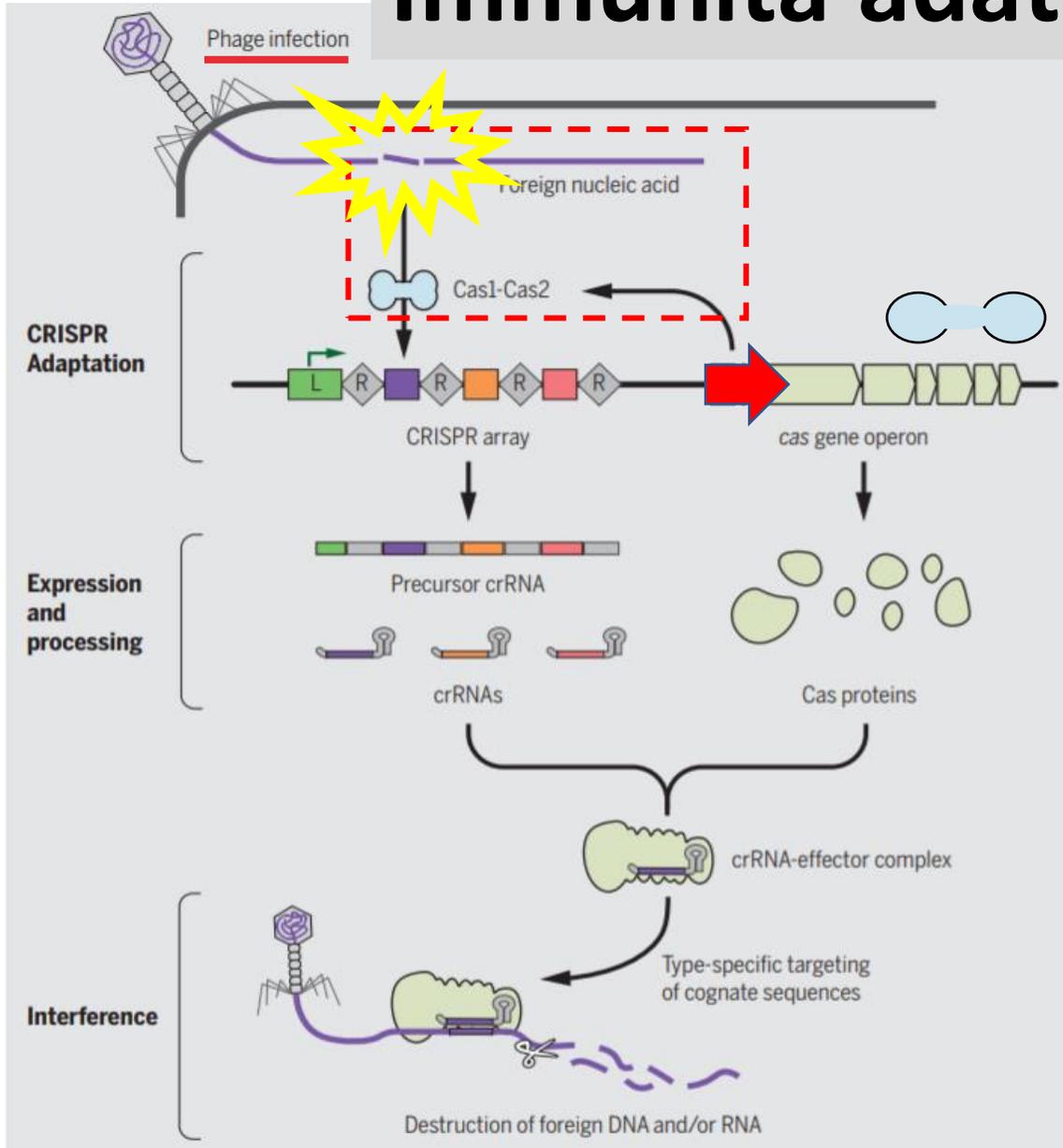
Struttura del locus CRISPR



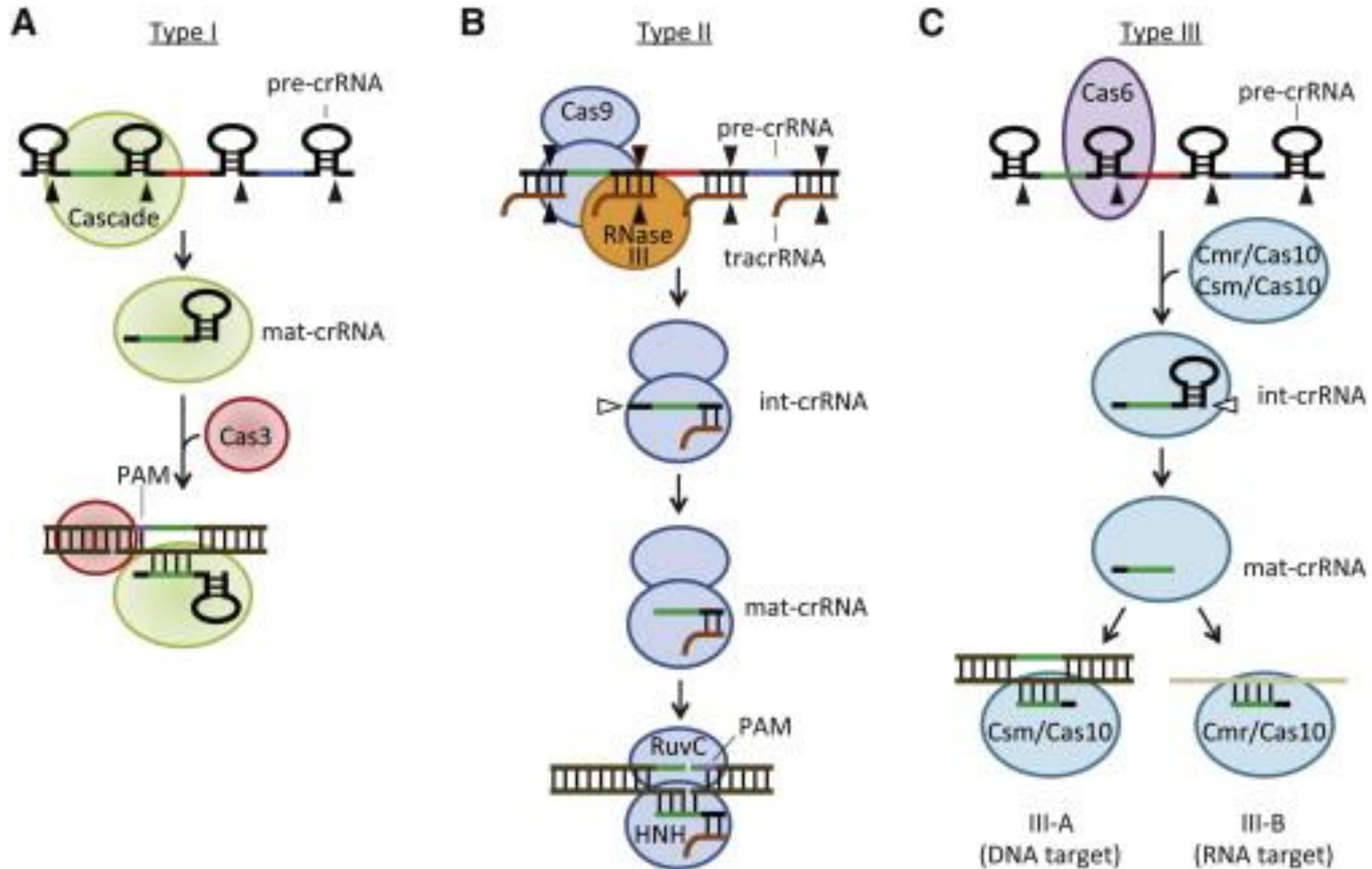
Immunità adattativa nei batteri



Immunità adattativa nei batteri



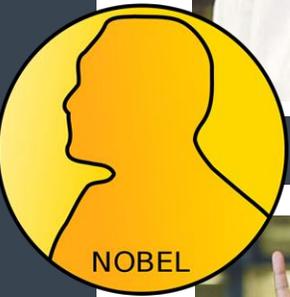
Immunità adattativa nei batteri



A 3D digital illustration of a CRISPR/Cas9 complex. The Cas9 protein is shown as a large, reddish, textured cluster. It is bound to a DNA double helix, which is highlighted in cyan and yellow. The surrounding DNA is shown in a lighter blue, semi-transparent style. The background is a dark, gradient blue with faint, repeating DNA helix patterns.

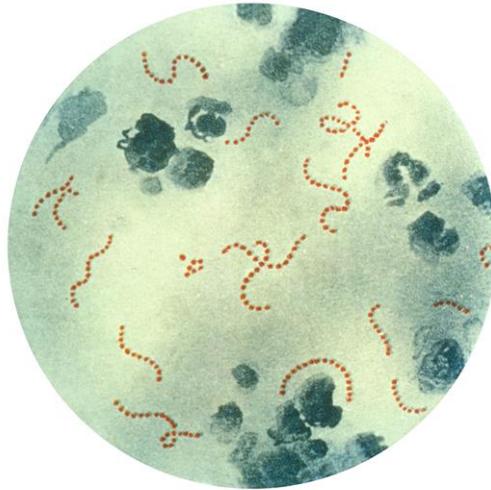
Genome editing con CRISPR/Cas9

Jennifer Doudna



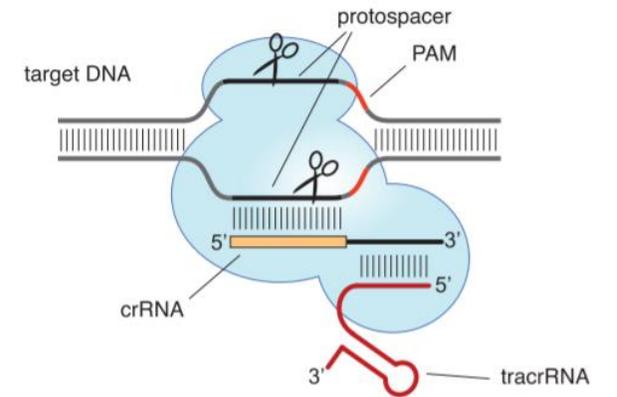
Emmanuelle Charpentier

Induzione della resistenza

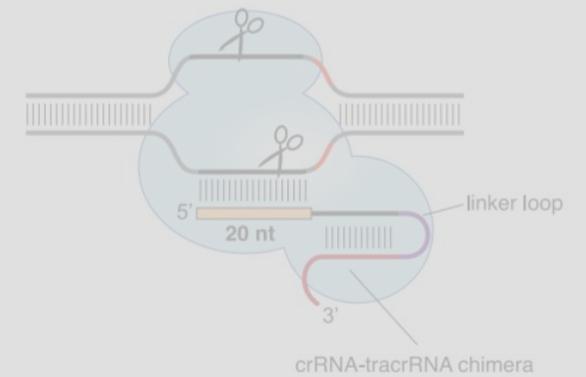


Streptococcus pyogenes

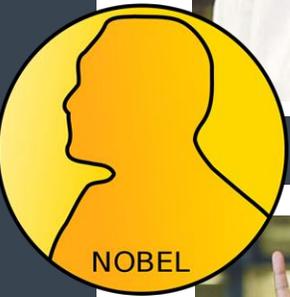
Cas9 programmed by crRNA:tracrRNA duplex



Cas9 programmed by single chimeric RNA

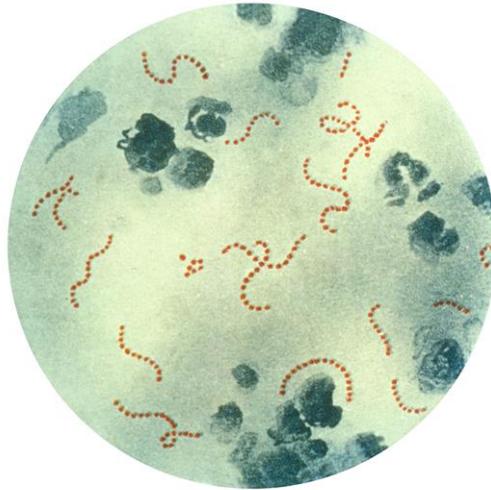


Jennifer Doudna



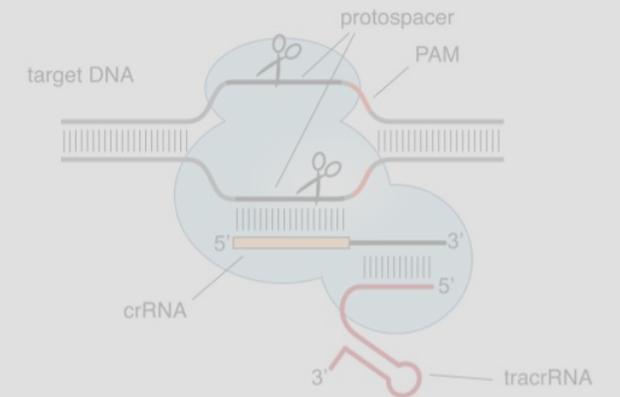
Emmanuelle Charpentier

Induzione della resistenza

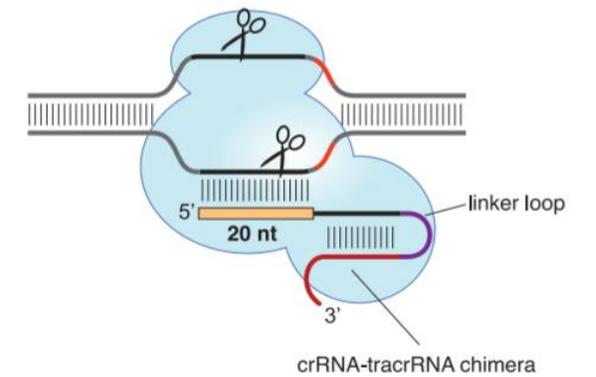


Streptococcus pyogenes

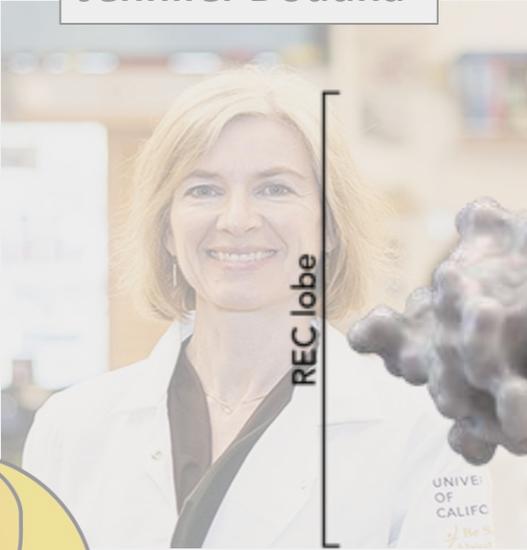
Cas9 programmed by crRNA:tracrRNA duplex



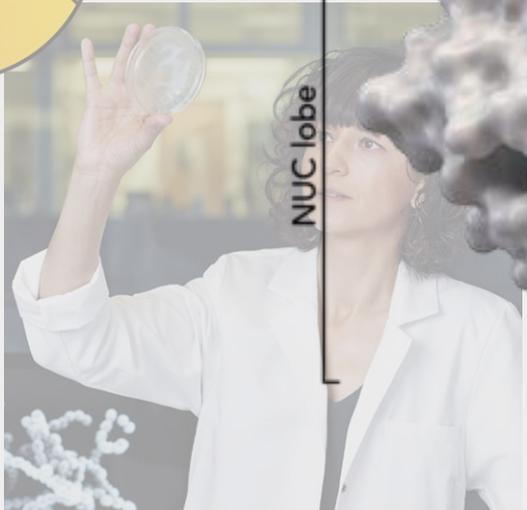
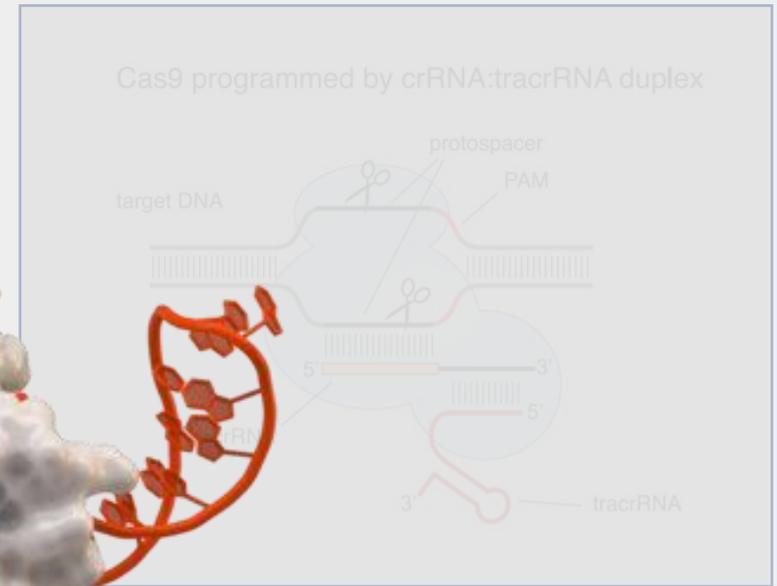
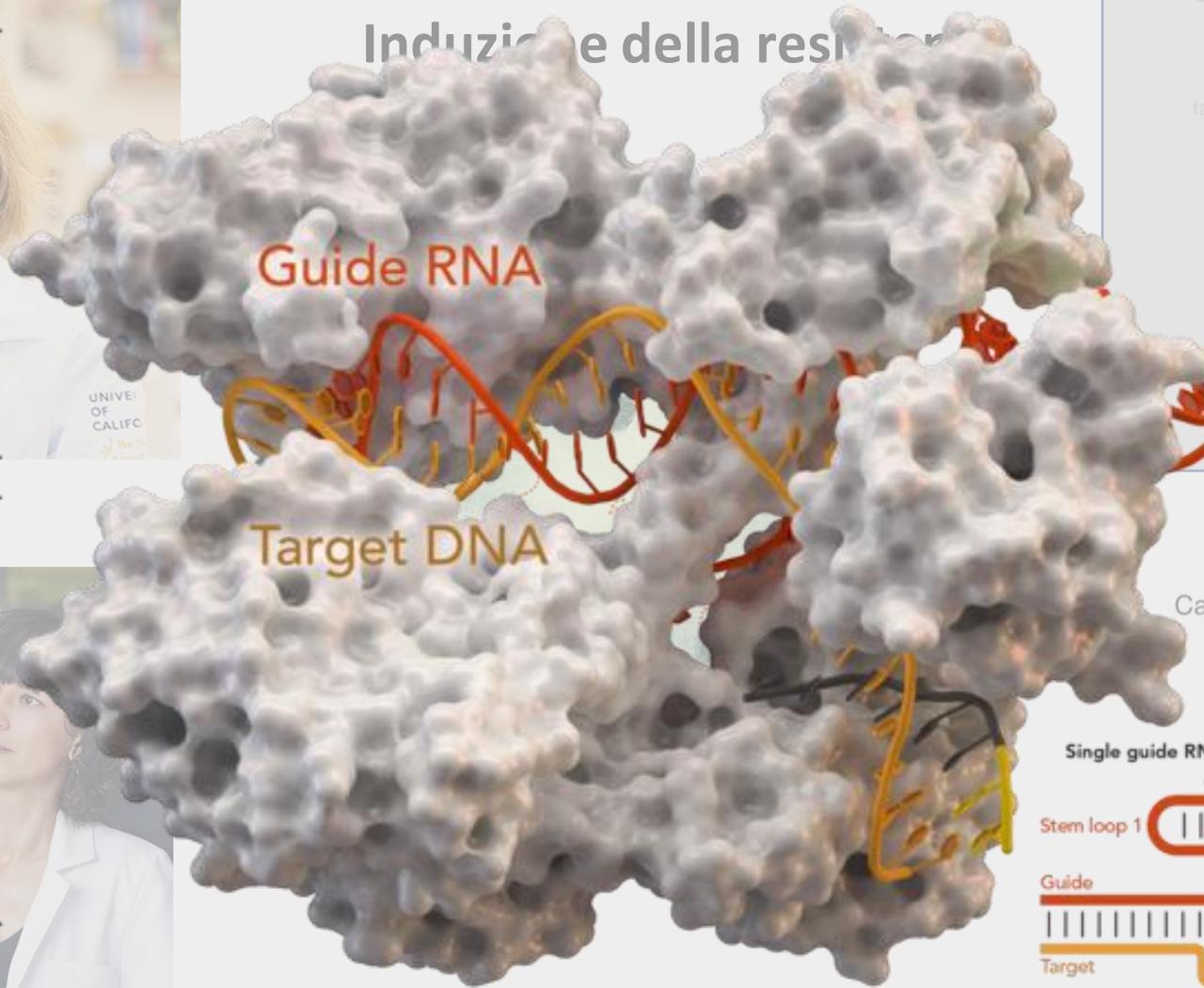
Cas9 programmed by single chimeric RNA



Jennifer Doudna

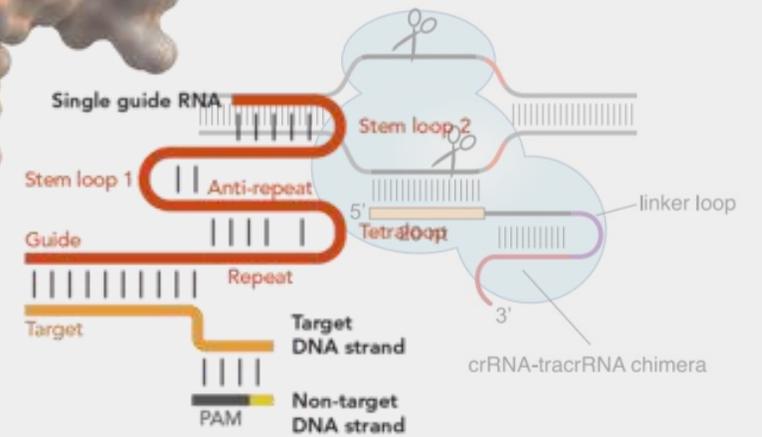


Induzione della resistenza

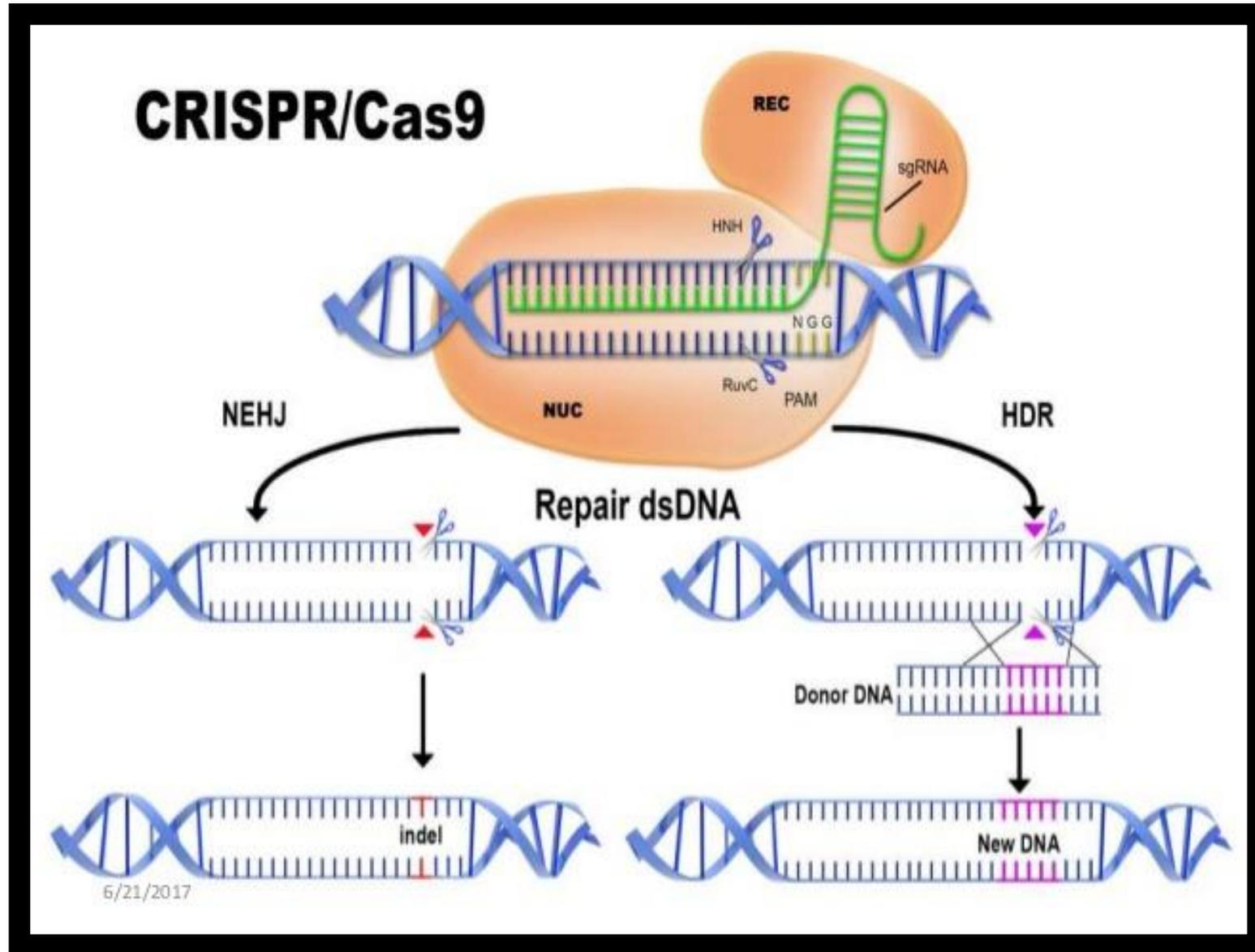


Emmanuelle Charpentier

Cas9 programmed by single chimeric RNA



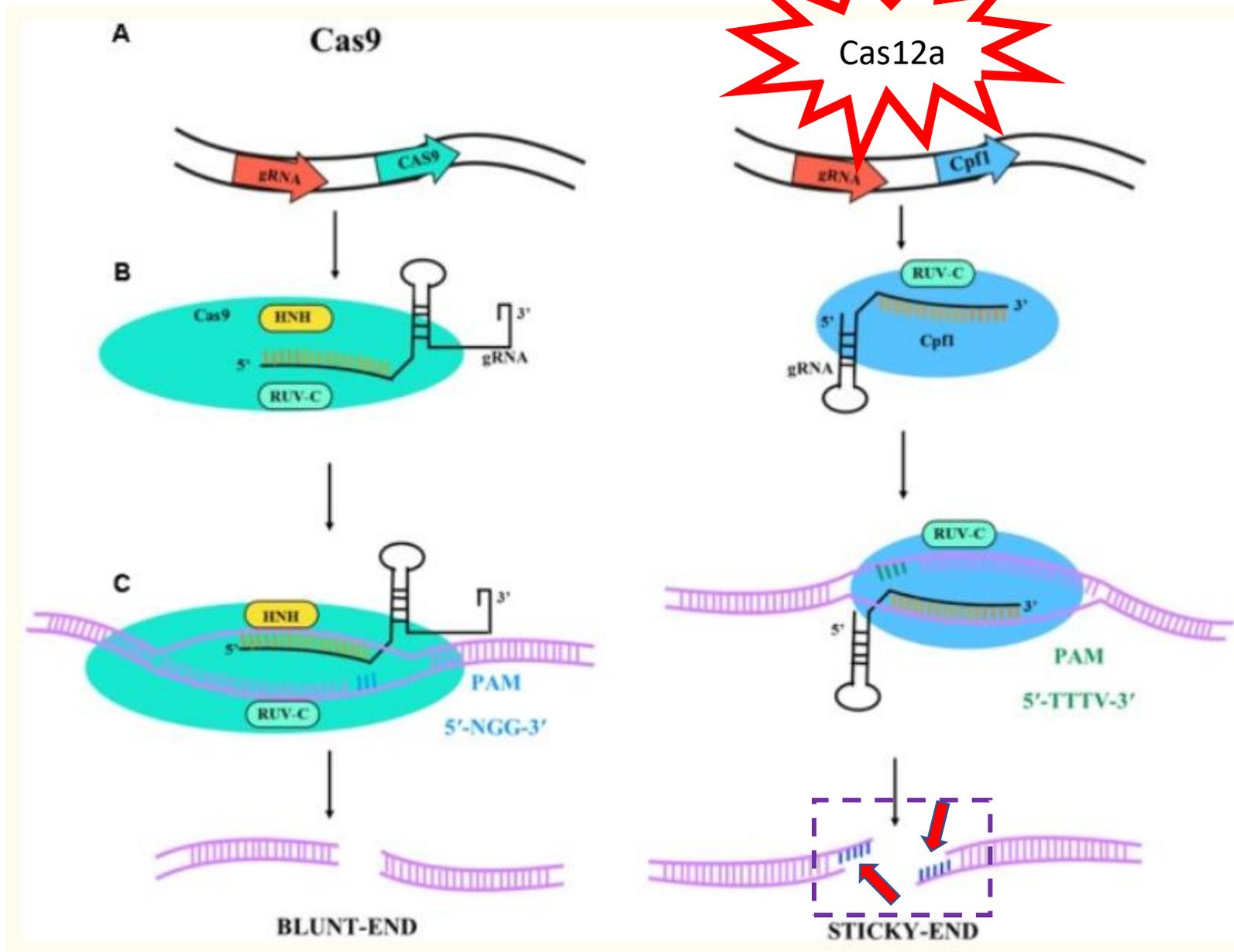
Genome editing con CRISPR/Cas9



Genome editing con CRISPR/Cas9



Genome editing con CRISPR/Cas12a



La formazione di estremità STICKY dall'evento di taglio permette di migliorare l'inserzione del gene di interesse

Danio rerio



Primate

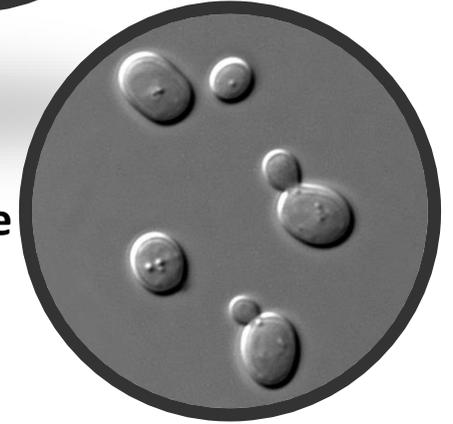


A. thaliana



D. melanogaster

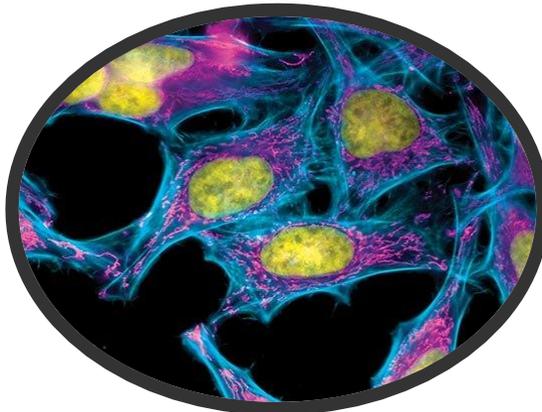
S. cerevisiae



Cavie



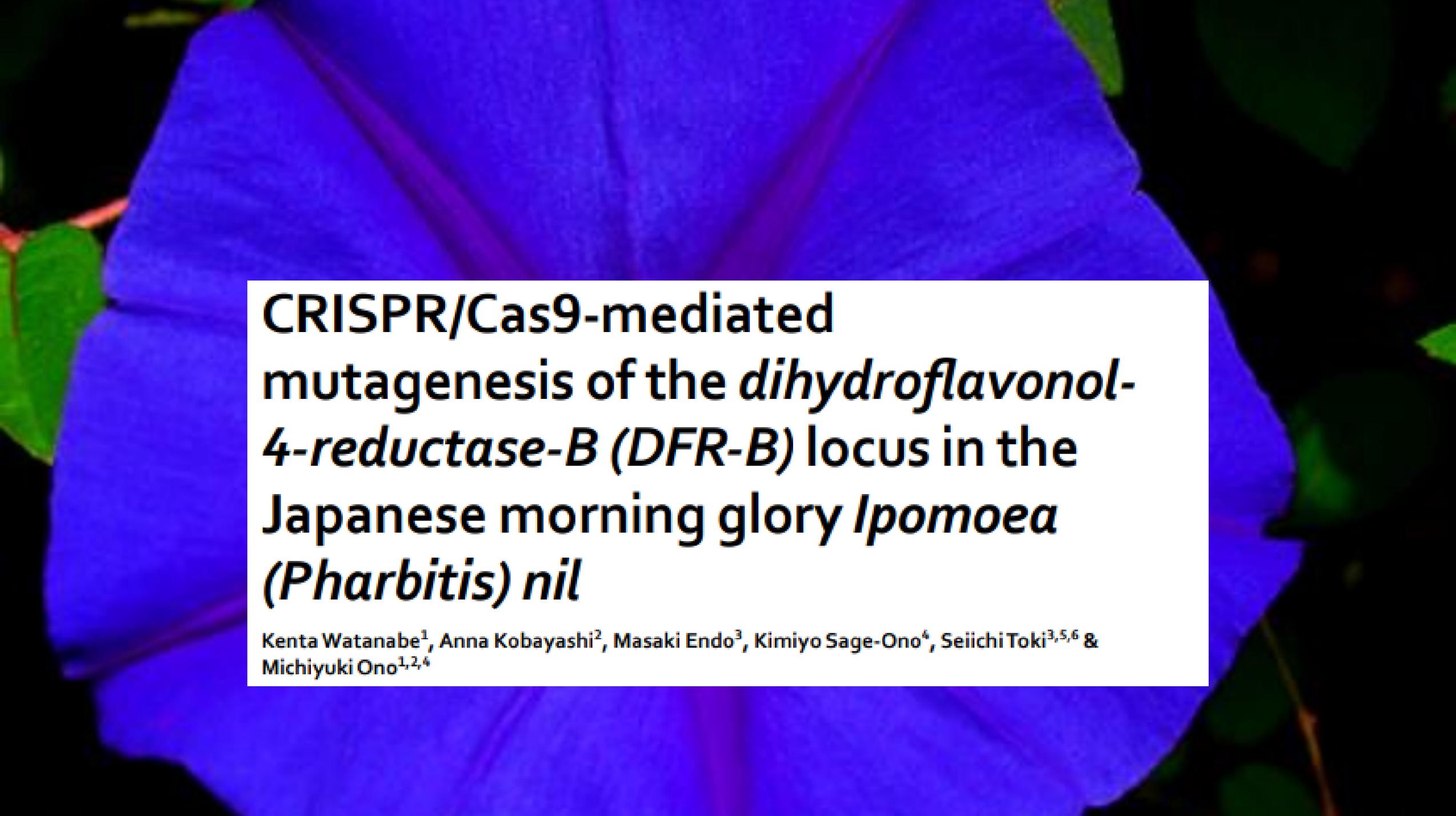
Cellule umane



C. elegans



**Genome editing con CRISPR/Cas9
negli organismi vegetali**



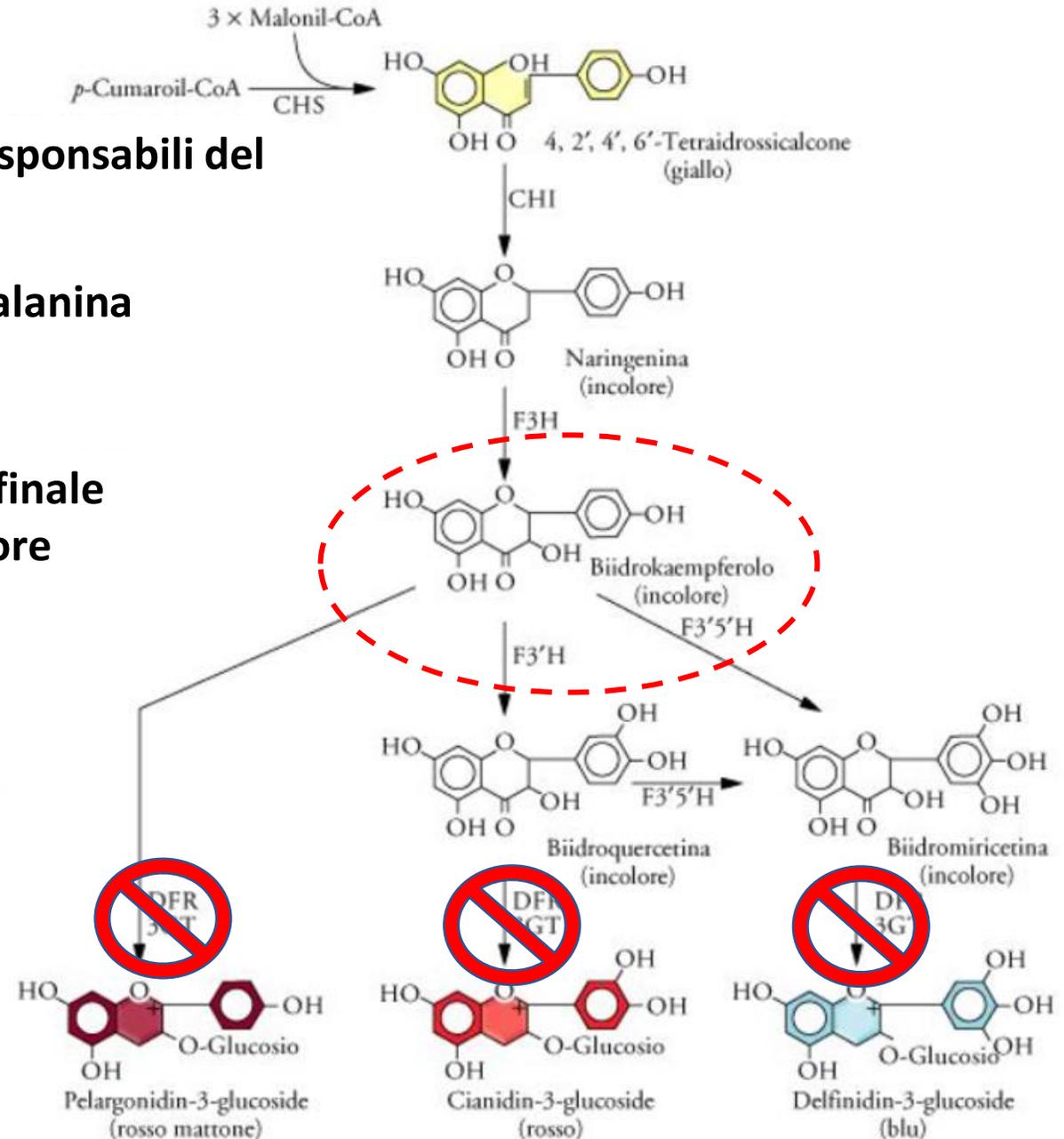
**CRISPR/Cas9-mediated
mutagenesis of the *dihydroflavonol-
4-reductase-B (DFR-B)* locus in the
Japanese morning glory *Ipomoea
(Pharbitis) nil***

Kenta Watanabe¹, Anna Kobayashi², Masaki Endo³, Kimiyo Sage-Ono⁴, Seiichi Toki^{3,5,6} & Michiyuki Ono^{1,2,4}

ANTOCIANINE: classe di flavonoidi responsabili del colore del fiore

Sono sintetizzate a partire dalla fenilalanina

Le catene laterali del prodotto finale determinano il pigmento del fiore





DFR-A: ...**C**AGCATCATACCACCAGT**A**CA...
DFR-B: ...AAGCATCATACCACCAGT**TGG**...
DFR-C: ...**CAGT**ATCATACCACCAGT**CA**...



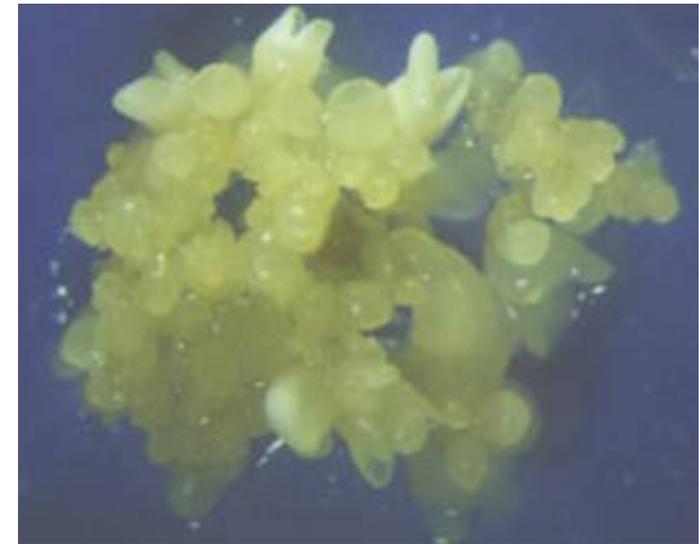


Ipomea nil cv Violet

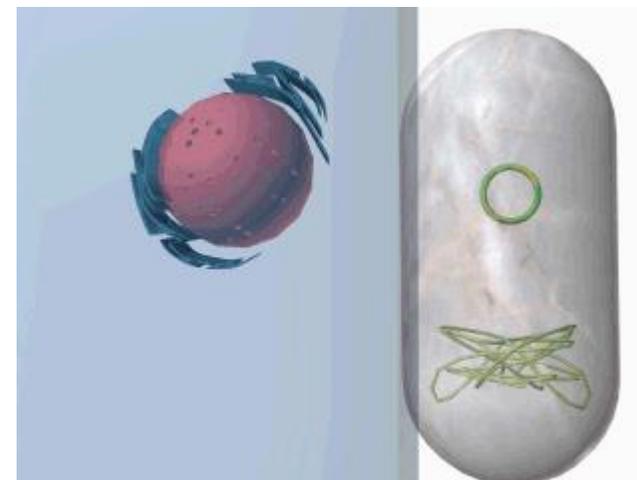
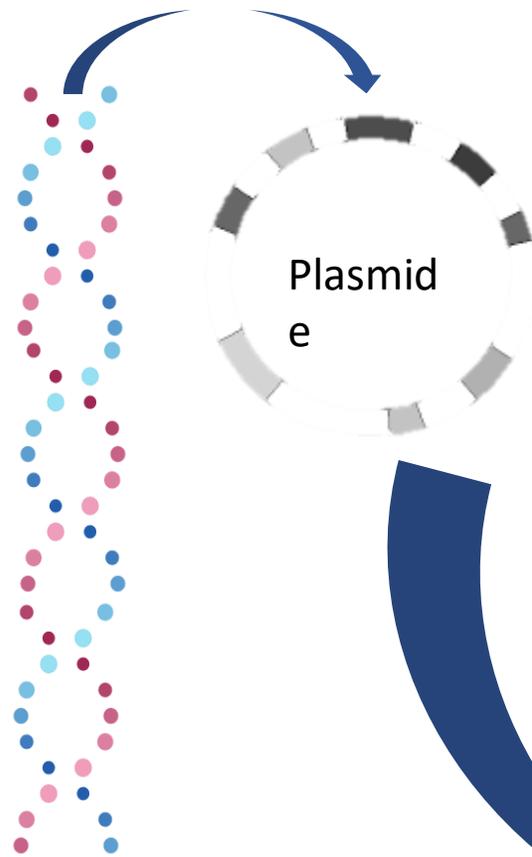
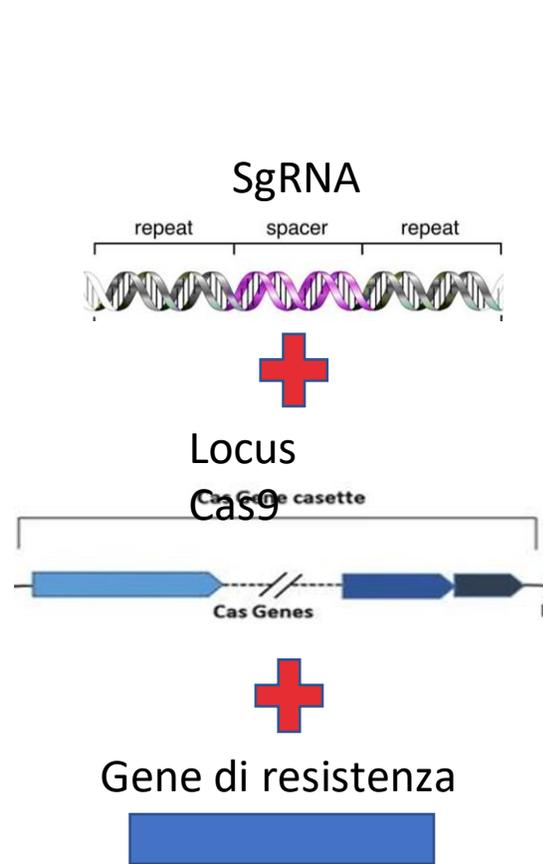


frutti immaturi

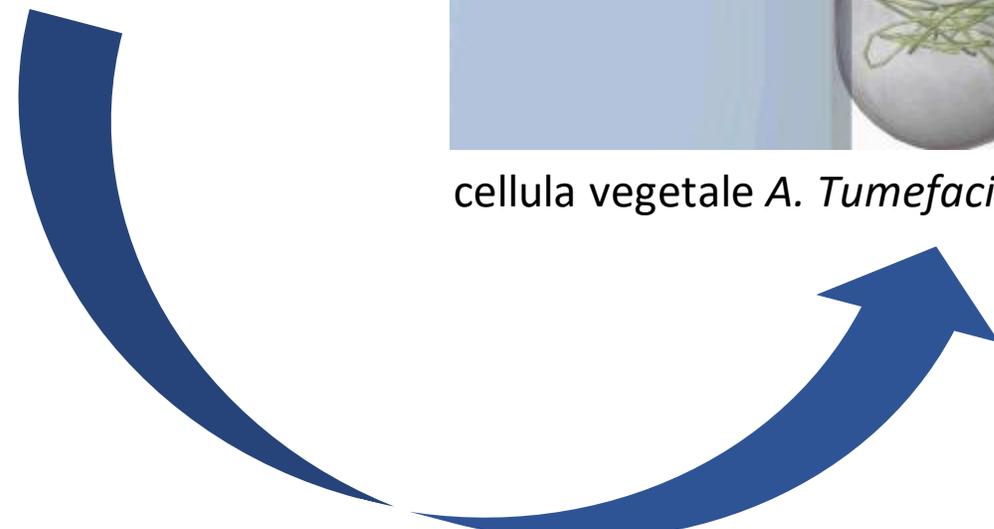
Sono stati sterilizzati e sono stati isolati gli
embrioni immaturi



Successivamente è stata eseguita la **trasformazione, mediata da *A. tumefaciens*** attraverso il vettore binario composto da:



cellula vegetale *A. Tumefaciens*



Mutagensi mirata mediata da CRISPR / Cas9 in DFR-B



violet stem

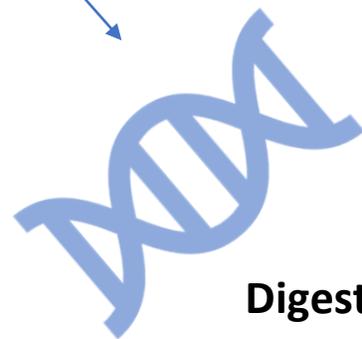
green stem

Mutagenesi mirata mediata da CRISPR / Cas9 in DFR-B

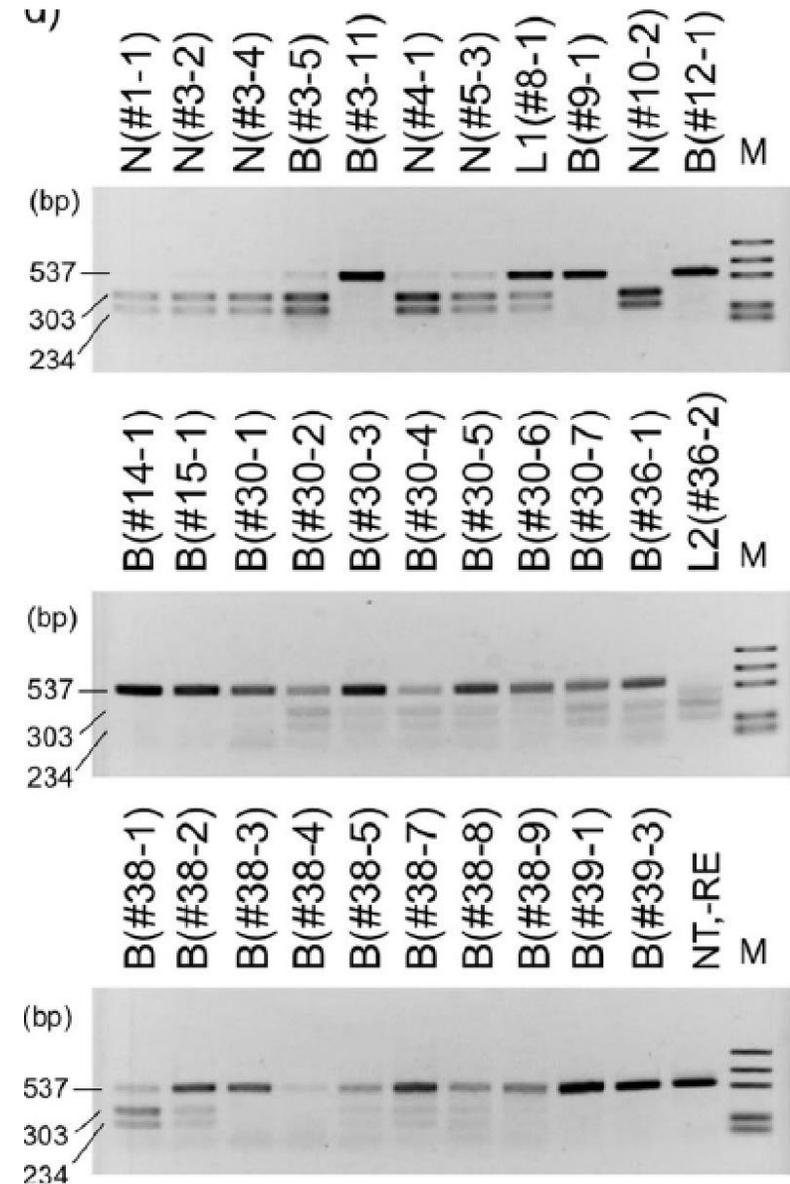


violet stem

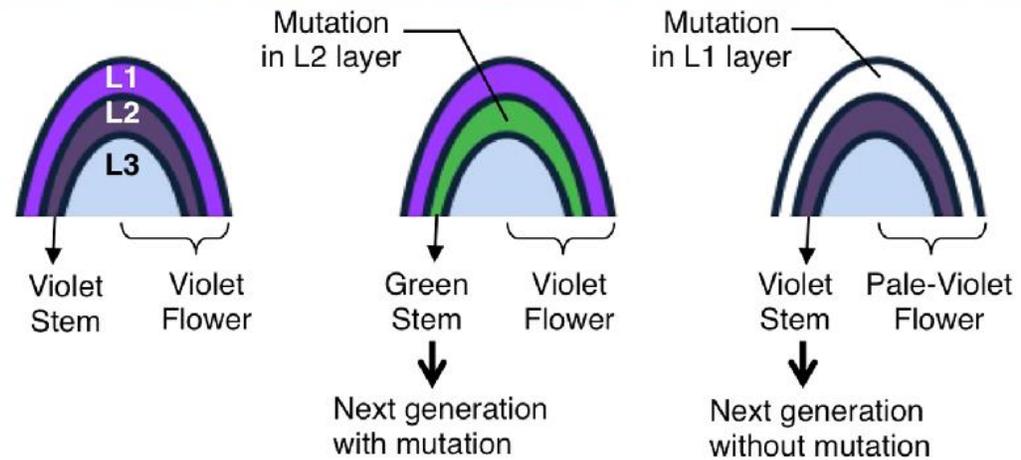
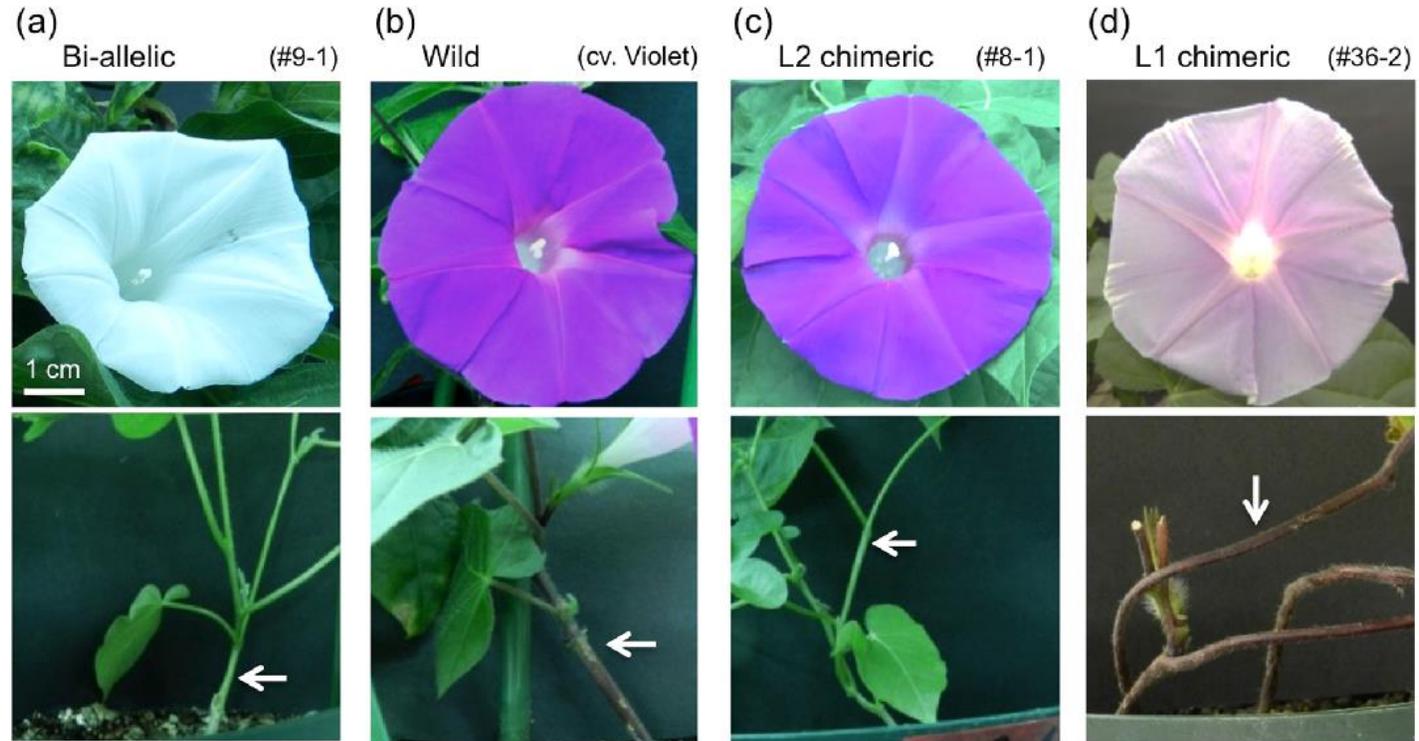
green stem



Digestione con SpeI



Mutagens mirata mediata da CRISPR / Cas9 in DFR-B



SCIENTIFIC REPORTS



OPEN

Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion

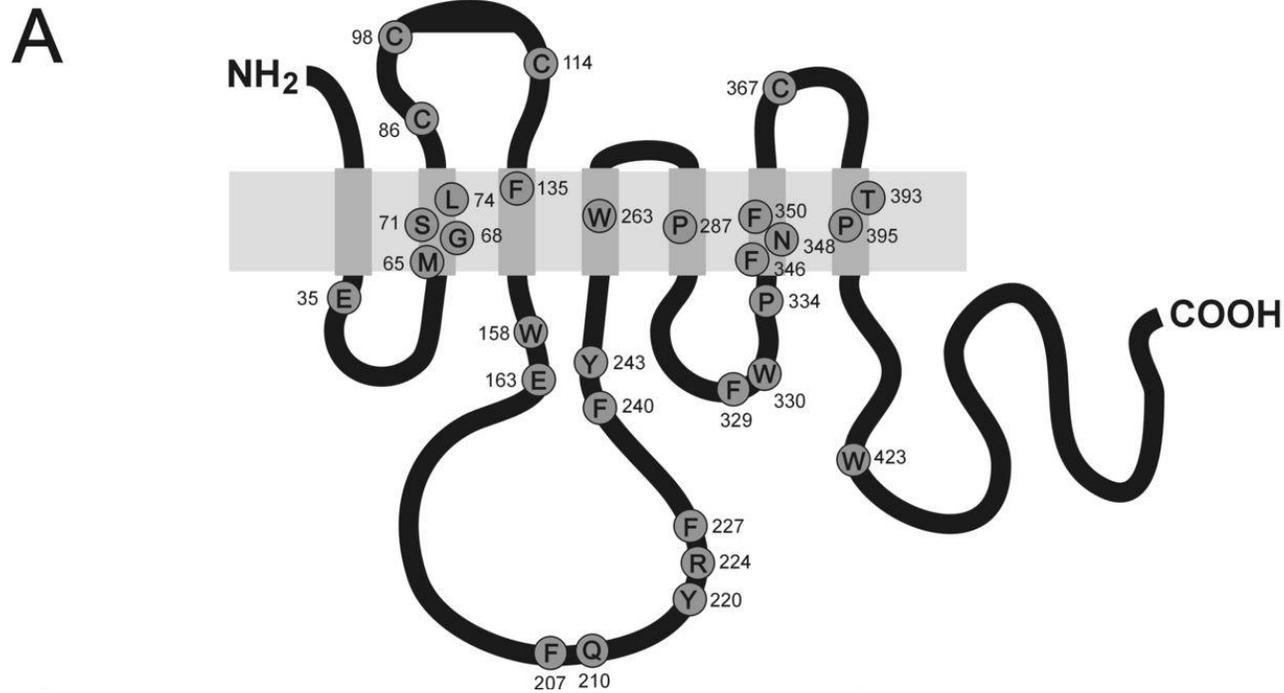
Received: 16 February 2017

Accepted: 22 February 2017

Published online: 28 March 2017

Vladimir Nekrasov^{1,4}, Congmao Wang², Joe Win¹, Christa Lanz³, Detlef Weigel³ & Sophien Kamoun ¹

Il locus *Mlo* (*MILDEW RESISTANT LOCUS O*) codifica proteine associate alla membrana, che presentano 7 domini tran-membrana. È un locus altamente conservato sia nelle monocotiledoni che nelle dicotiledoni.



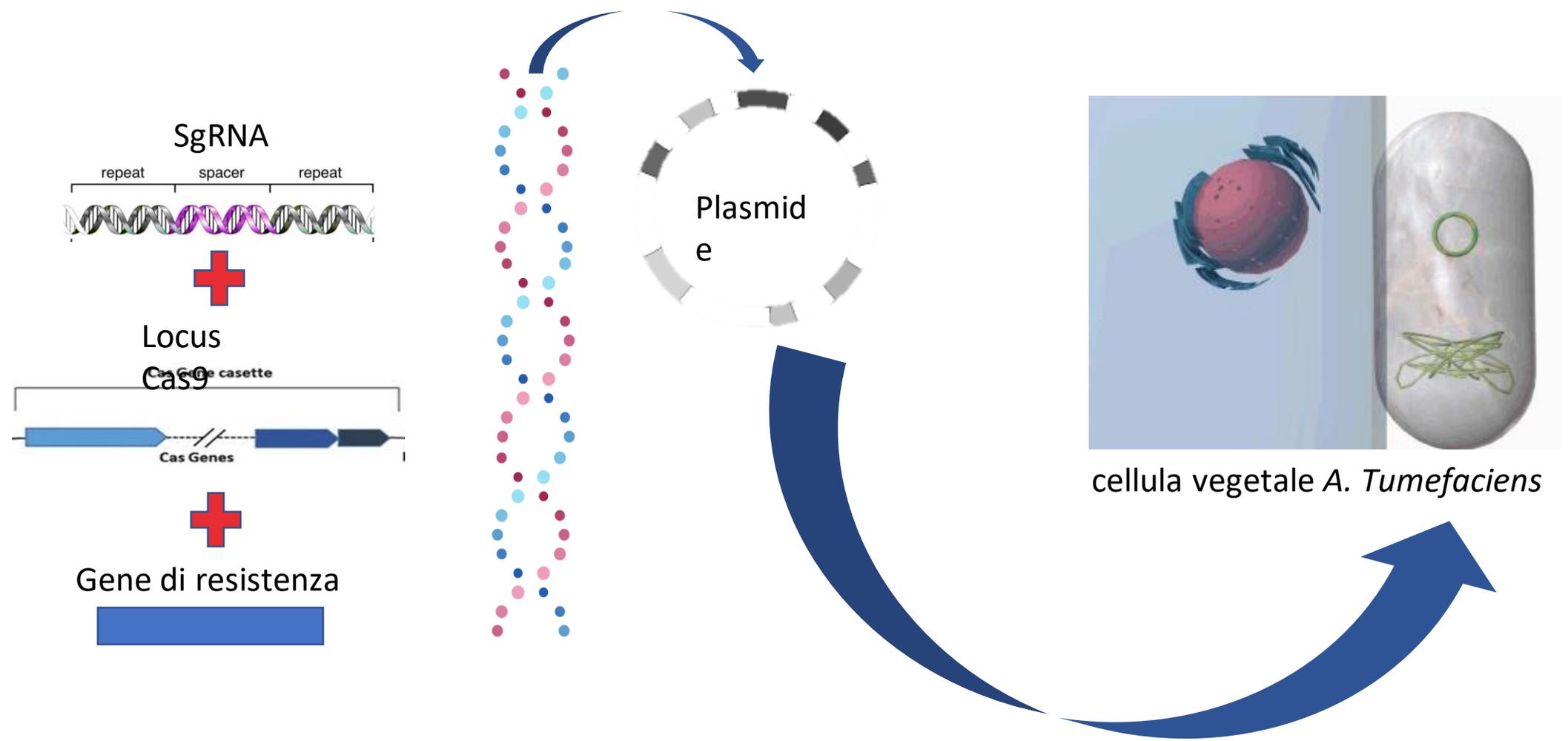
Questo locus conferisce suscettibilità ai funghi in particolare alla malattia dell'oidio, il mal bianco



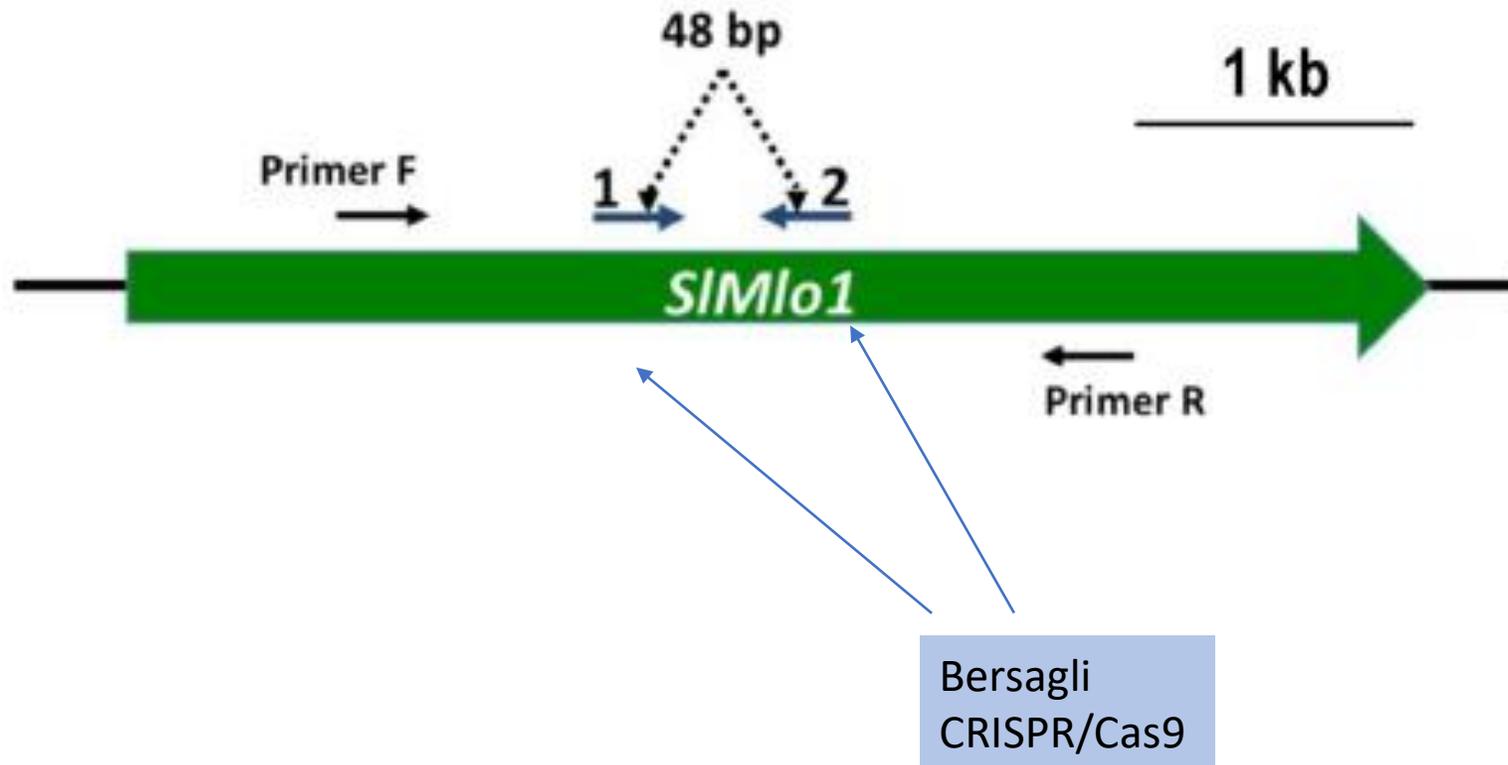
Mutazioni in omozigosi con perdita di funzione (*mlo*) risultano in piante resistenti all'oidio.

Nel pomodoro, ci sono 16 geni *Mlo* nel locus, da *SIMlo1* a *SIMlo16*.

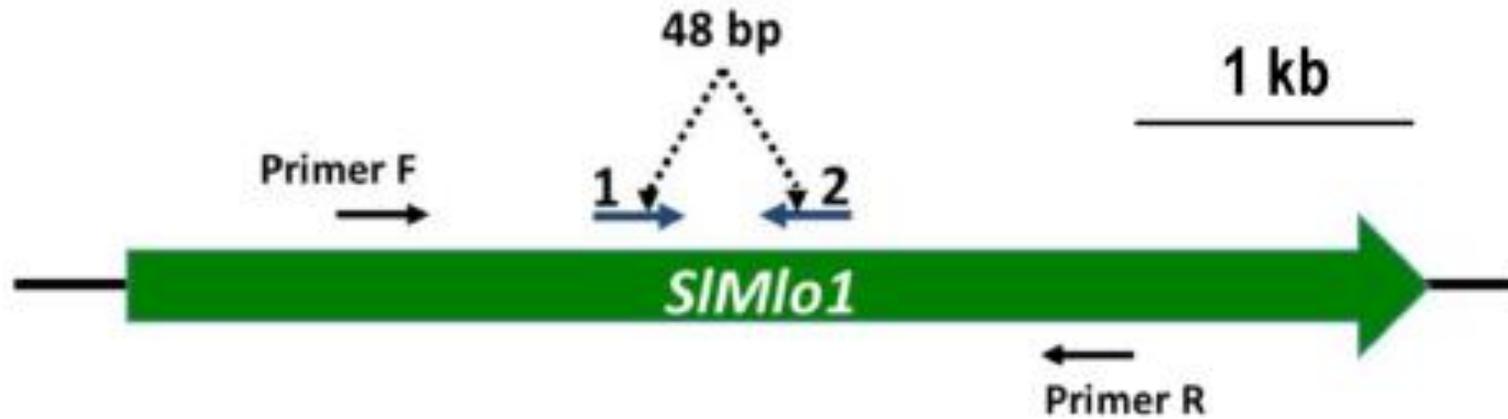
Successivamente è stata eseguita la **trasformazione, mediata da *A. tumefaciens*** attraverso il vettore binario composto da:



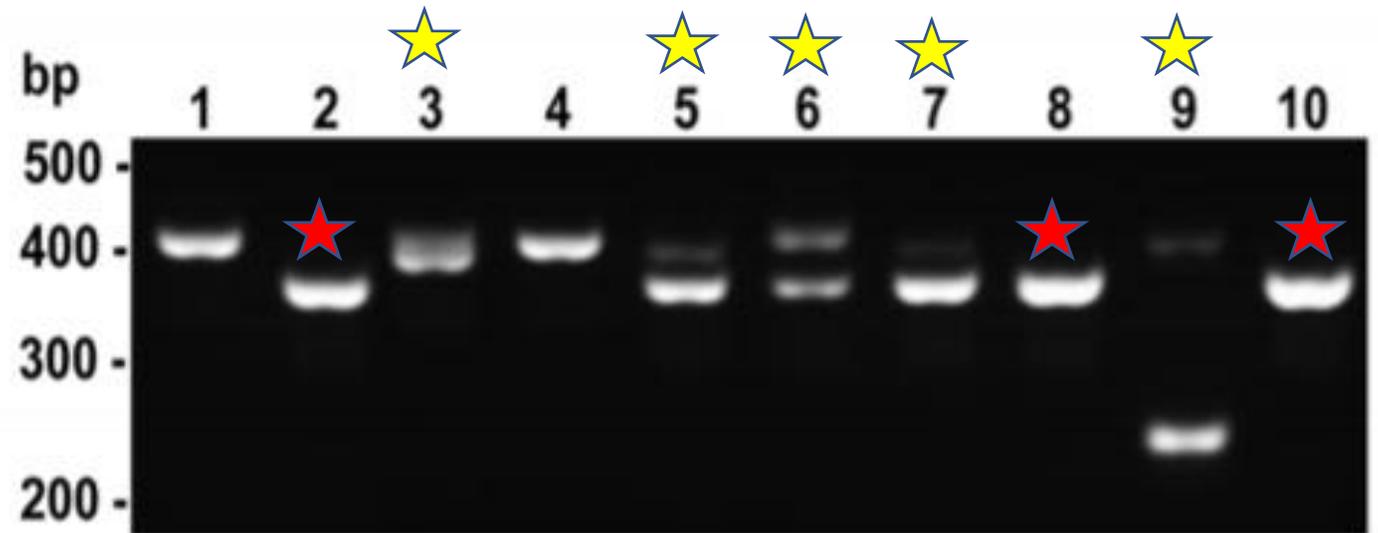
Dimostrare la possibilità di generare delle varietà di pomodoro , di interesse economico , con **la mutazione *slmlo1*** usando il sistema CRISPR / Cas9 ed evitando la classificazione come piante transgeniche.

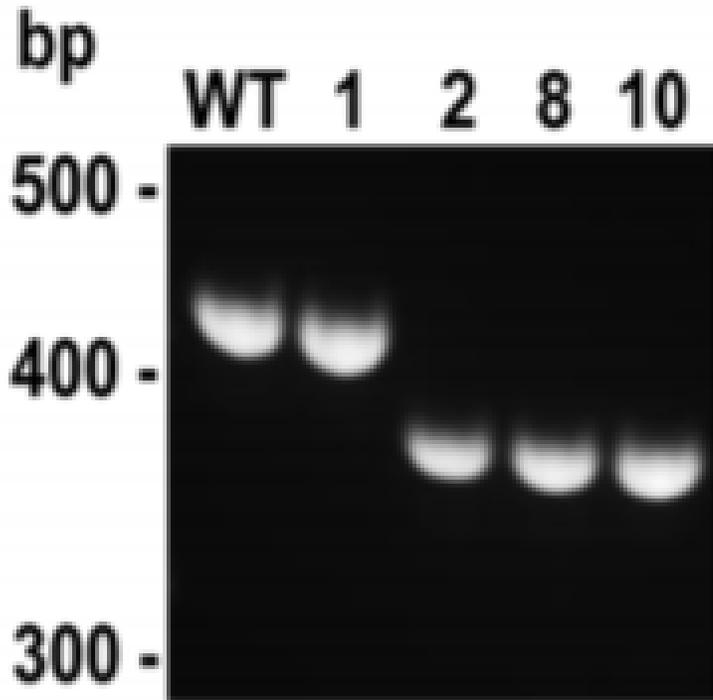


Dimostrare la possibilità di generare delle varietà di pomodoro , di interesse economico , con **la mutazione *slmlo1*** usando il sistema CRISPR / Cas9 ed evitando la classificazione come piante transgeniche.



Otto trasformati testati su dieci hanno mostrato la presenza di mutazioni



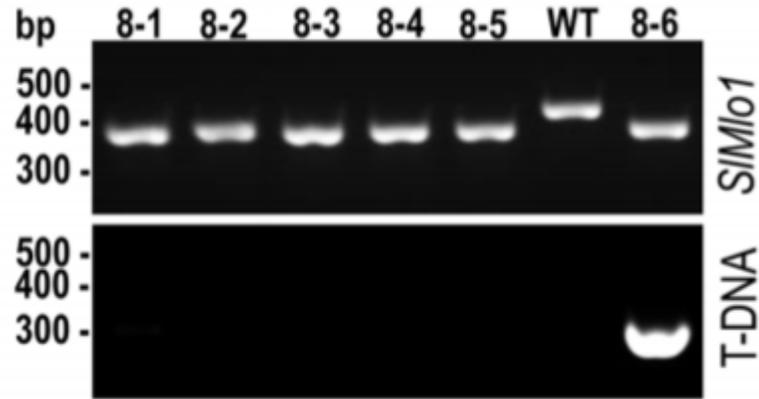


Sono stati selezionati tre trasformanti
2, 8 e 10 con la delezione prevista di 48bp

d

	Target 1	PAM	PAM	Target 2	
WT	ACATAGTAAAA GGTGTACCTGTGGTGGAGAC	<u>TGGTGACC</u> ATCTTTTCTGGTTTAATCGCCCTGCCCTTGTCCCT	<u>ATTCTTGATTA</u> ACTTTGTACTCTTTCAGG		
Plant 1	ACATAGTAAAA GGTGTACCTGTGGTGGAGAC	TGGTGACC	ATCTTGATTA	ACTTTGTACTCTTTCAGG	
Plant 2	ACATAGTAAAA GGTGTACCTGTGGTGGG	-----	-----	CTTGATTA ACTTTGTACTCTTTCAGG	-48
Plant 8	ACATAGTAAAA GGTGTACCTGTGGTGGG	-----	-----	CTTGATTA ACTTTGTACTCTTTCAGG	-48
Plant 10	ACATAGTAAAA GGTGTACCTGTGGTGGG	-----	-----	CTTGATTA ACTTTGTACTCTTTCAGG	-48
	ACATAGTAAAA GGTGTACCTGTGGTGGG	-----	-----	TTGATTA ACTTTGTACTCTTTCAGG	-49

Eliminazione del T-DNA



CRISPR/CAS9

- **Può provocare mutazioni indistinguibili da quelle spontanee**
- **Permette di controllare il sito di inserzione di un gene di interesse**
- **Efficiente**
- **Preciso**

NUC lobe
NUC lobe

