THE SAINSBURY LABORATORY

CRISPR Crops —
Plant Genome Editing Made Easy

@KamounLab

http://KamounLab.net

GATSBY

erc

erc

BBSRC
bioscience for the future

BASF
We create chemistry

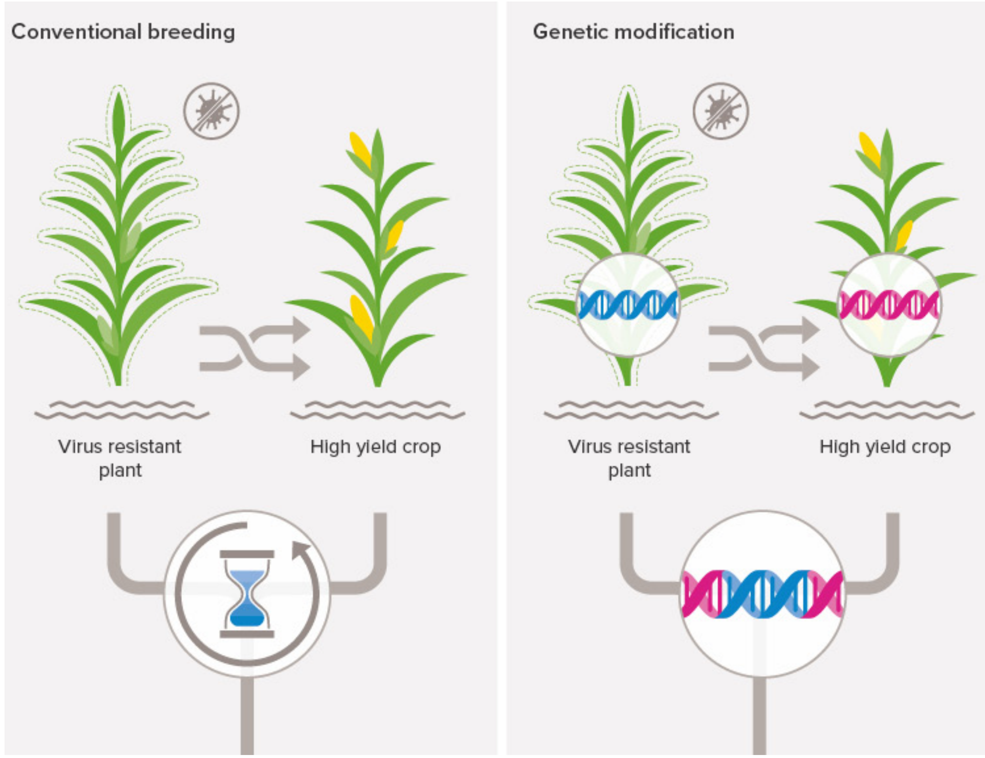
RIJK ZWAAN

Today's outline

- What is CRISPR?
- Applications of CRISPR gene editing
- CRISPR and conservation biology

Genetic improvement of plants

- Hybridization
- Introgression
- Mutagenesis
- Transgenics



Gene editing – the ultimate in precision is to replace one of a few letters in the genome

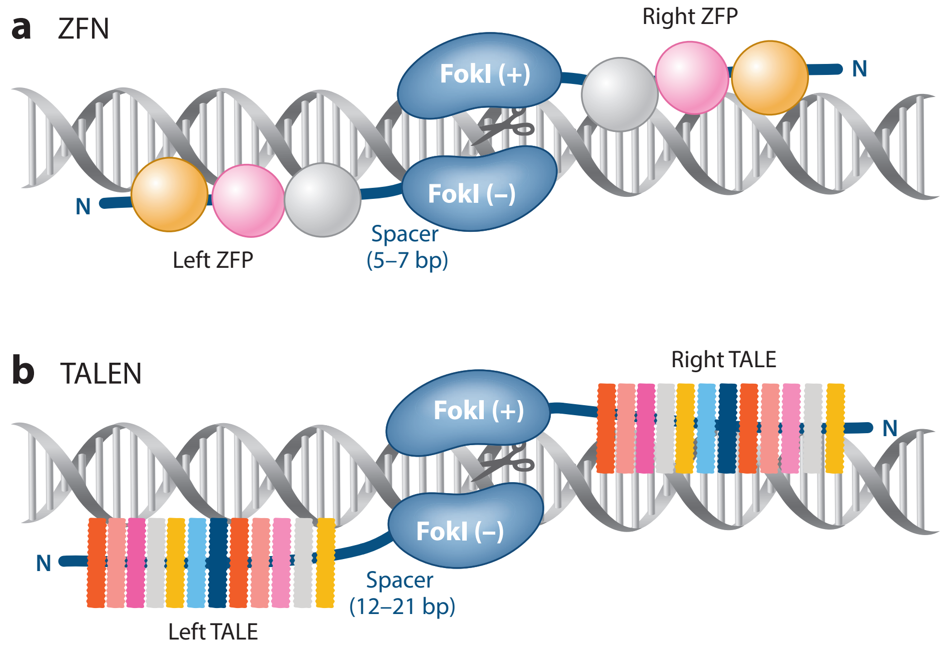
Reverse genetics in plants *circa* ~2012

- Genome sequencing of mutagenized plants
- TILLING — targeted sequencing of mutant plants
- T-DNA or transposon mutagenesis
- Gene knock-down using RNAi (VIGS, artificial miRNA, hairpin constructs)
- Site-specific nucleases (e.g. ZFNs and TALENs) for gene editing

Sequence-specific nucleases (SSNs)

- Zinc-Finger Nucleases

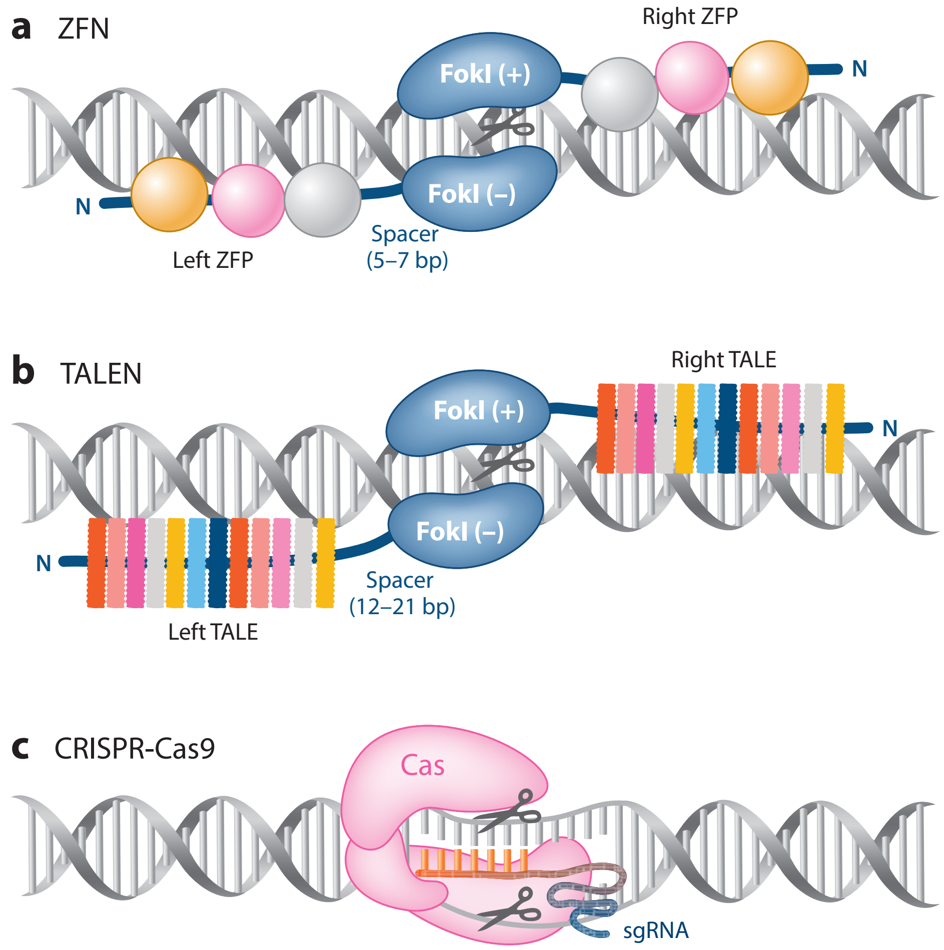
- TAL-Effector Nucleases



Langner et al. 2018

Sequence-specific nucleases (SSNs)

- Zinc-Finger Nucleases
- TAL-Effector Nucleases
- CRISPR/Cas9



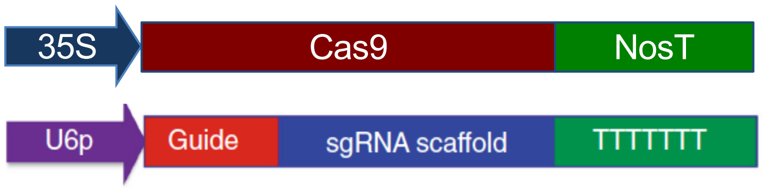
Langner et al. 2018

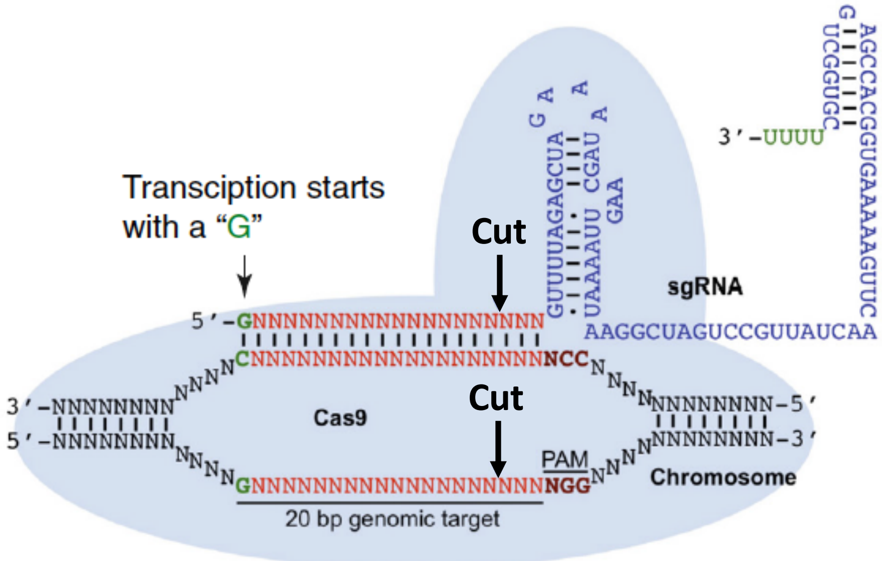
The CRISPR Craze

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]‡

Cas9 and sgRNA are the two components of the CRISPR system





The New York Times

Opinion

This Year's Nobel Prize in Chemistry Honors a Revolution

With Crispr, two scientists turned a curiosity of nature into an invention that will transform the human race.

The CRISPR Craze

Sciencexpress Report

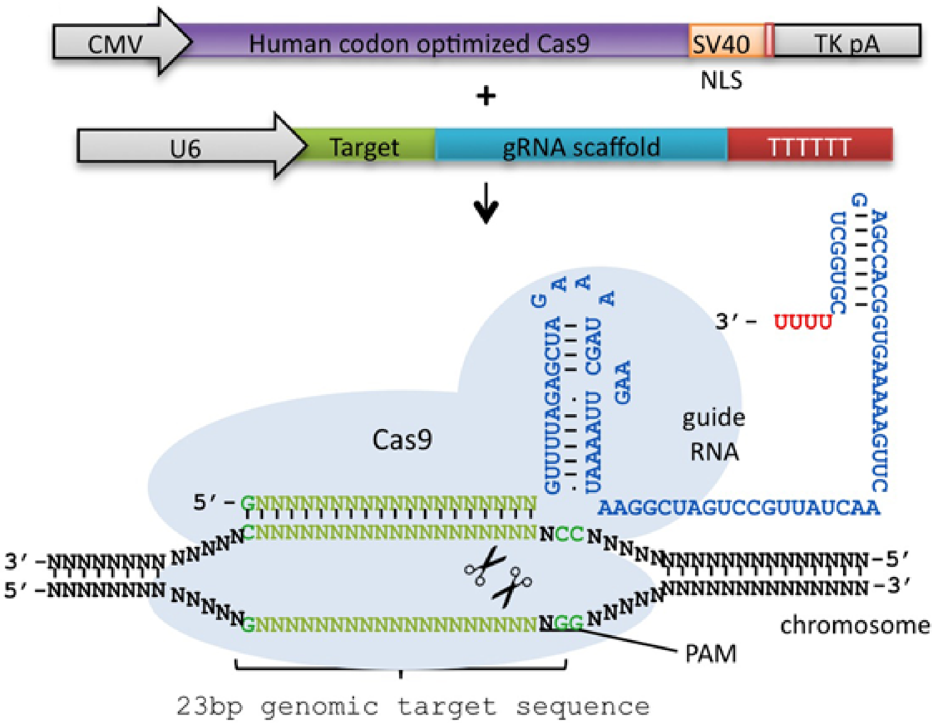
03 January 2013

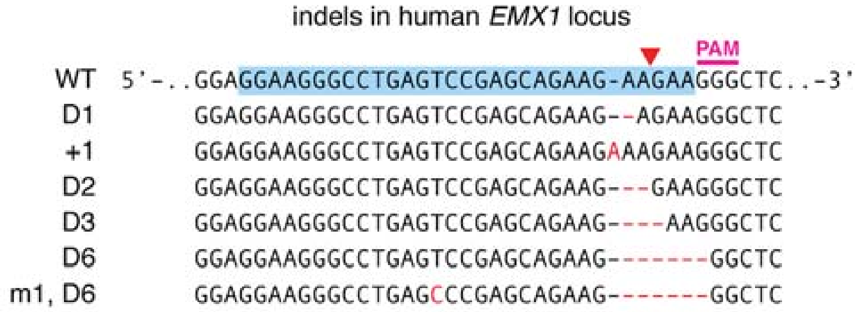
RNA-Guided Human Genome Engineering via Cas9

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] George M. Church[1,2*]

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 Marraffini,[8] Feng Zhang[1]†





The CRISPR Craze

infects plant scientists

VOLUME 31 NUMBER 8 AUGUST 2013 NATURE BIOTECHNOLOGY

Multiplex and homologous recombination–mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9

Jian-Feng Li[1,2], Julie E Norville[2,3], John Aach[2,3], Matthew McCormack[1,2], Dandan Zhang[1,2], Jenifer Bush[1,2], George M Church[2,3] & Jen Sheen[1,2]

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

Qiwei Shan[1,4], Yanpeng Wang[1,4], Jun Li[1,4], Yi Zhang[1], Kunling Chen[1], Zhen Liang[1], Kang Zhang[1], Jinxing Liu[1], Jianzhong Jeff Xi[2], Jin-Long Qiu[3] & Caixia Gao[1]

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

Vladimir Nekrasov[1], Brian Staskawicz[2], Detlef Weigel[3], Jonathan D G Jones[1,4] & Sophien Kamoun[1,4]

Open
LETTER TO THE EDITOR

Cell Research (2013) :1-4.

www.nature.com/cr

npg

Efficient genome editing in plants using a CRISPR/Cas system

Cell Research advance online publication 20 August 2013; doi:10.1038/cr.2013.114

Zhengyan Feng[1, 2, 3, *], Botao Zhang[1, *], Wona Ding[4],
Xiaod
Fengqi
Yanfei

SCIENCE VOL 341 23 AUGUST 2013

Open
LETTER TO

Target

Cell Research

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013

Kabın Xıe and Yınong Yang*

Nucleic Acids Research, 2013, 1–12
doi:10.1093/nar/gkt780

Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice

Wenzhi Jiang[1], Huanbin Zhou[2], Honghao Bi[2], Michael Fromm[3], Bing Yang[2] and Donald P. Weeks[1,*]

G3: Genes|Genomes|Genetics Early Online, published on October 11, 2013

RNA guided genome editing for target gene mutations in wheat

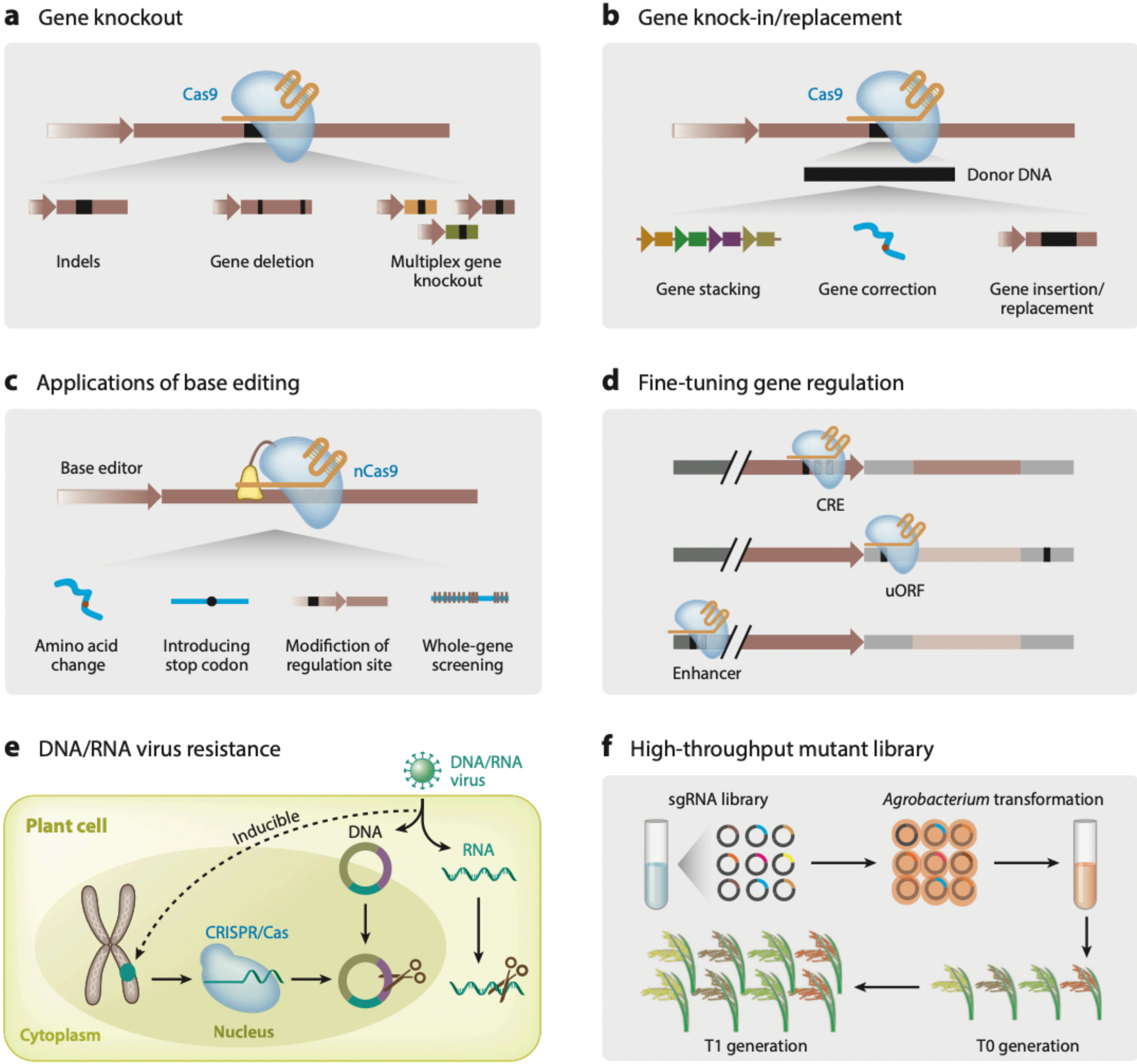
Santosh Kumar Upadhyay, Jitesh Kumar, Anshu Alok, RakeshTuli

Annual Review of Plant Biology

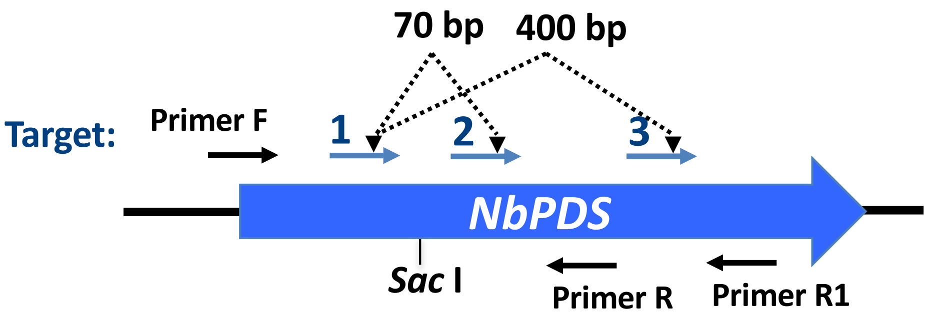
CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture

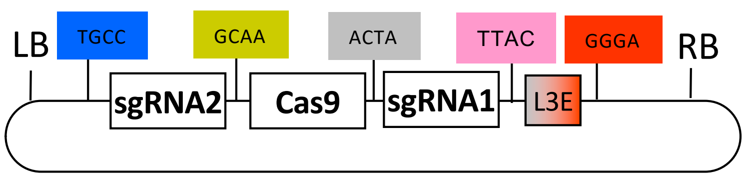
Kunling Chen,[1,*] Yanpeng Wang,[1,*] Rui Zhang,[1] Huawei Zhang,[1] and Caixia Gao[1,2]

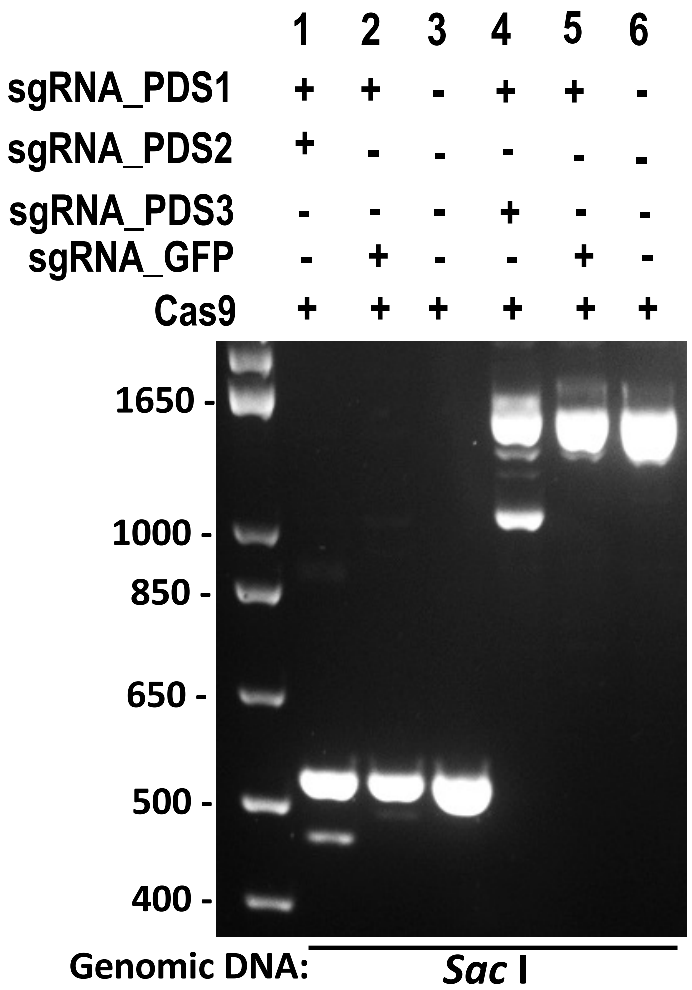
What can be done with CRISPR?



CRISPR/Cas9 enables making *small* deletions…



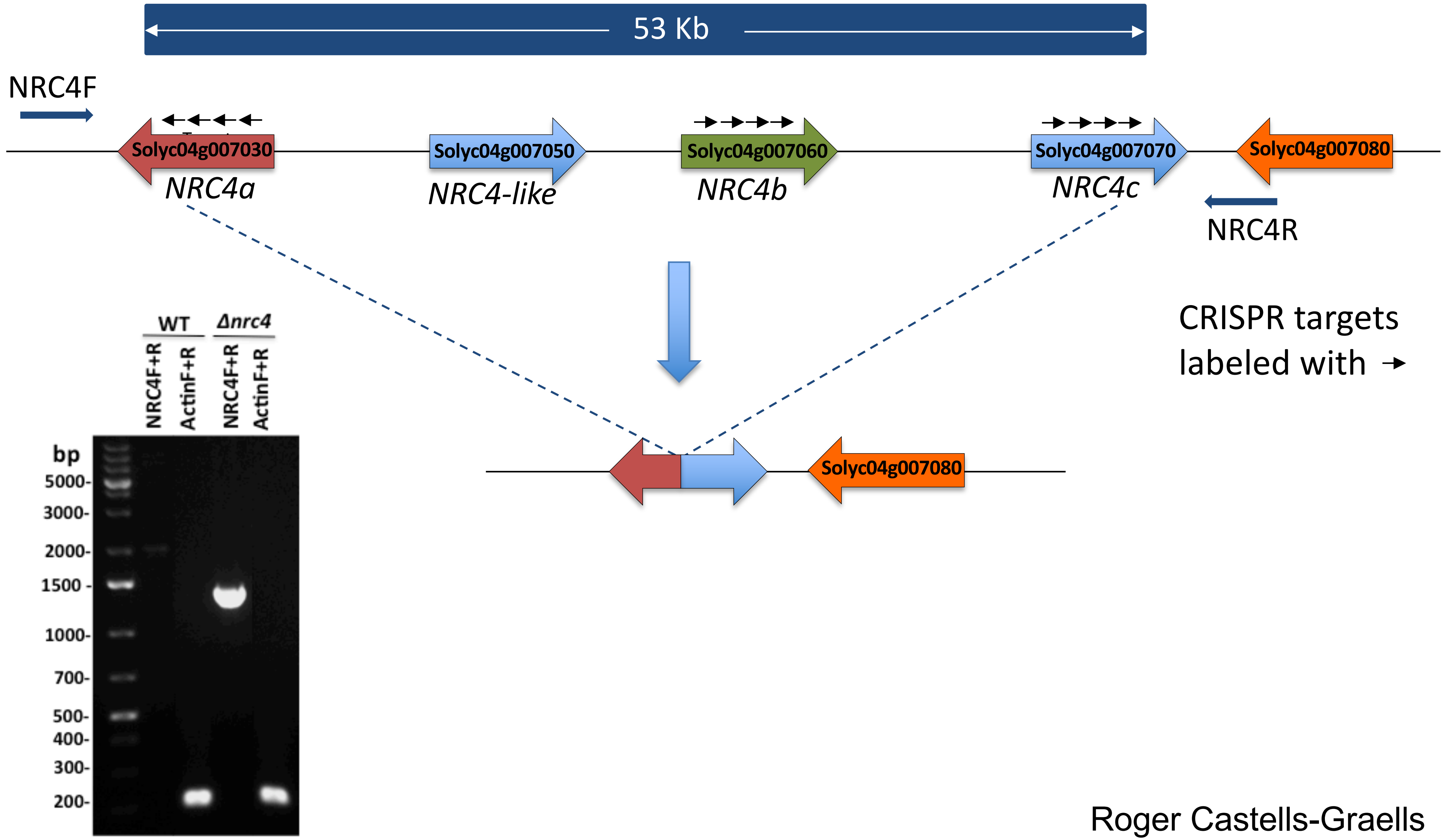





```
              Target 1          PAM                                                                     Target 2        PAM
PDS  TTCTGCCGTTAATTTGAGAGT-CCAAGGTAATTCAGCTTATCTTTGGAGCTCGAGGTCTTCGTTGGGAACTGAAAGTCAAGATGTTTGCTTGCAAAGGAATT
m1   TTCTGCCGTTAATTT------------------------------------------------------------------------------CAAAGGAATT -76
m2   TTCTGCCGTTAATTTGAGAGT----------------------------------------------------------------------CAAAGGAATT -70
m3   TTCTGCCGTTAATTTGAGAGTT---------------------------------------------------------------------CAAAGGAATT -70 +1 T
```

Belhaj *et al. Plant Methods* 2013, **9**:39

…and *BIG* deletions



Roger Castells-Graells
Vladimir Nekrasov

Annual Review of Plant Biology

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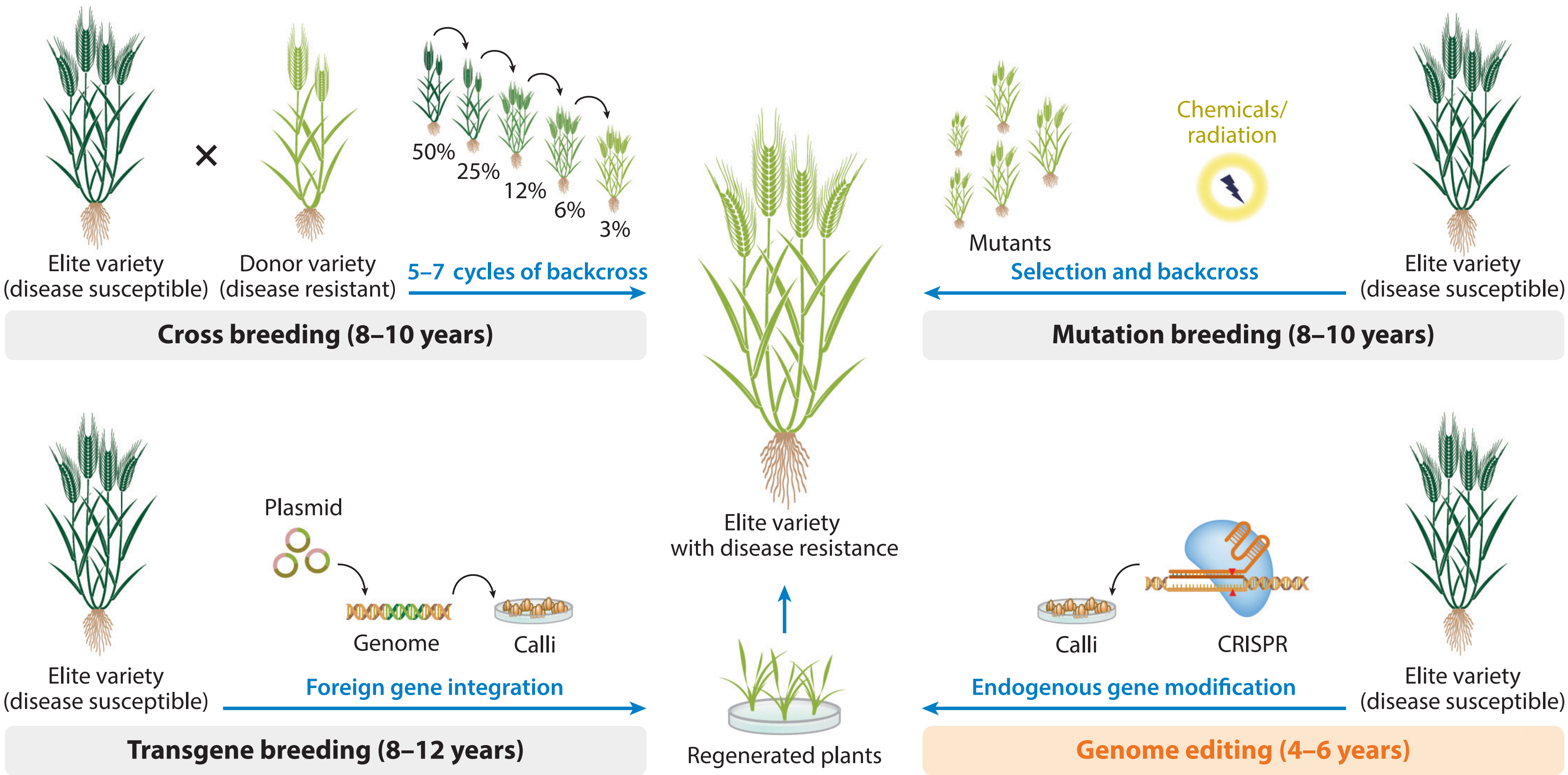


Crop plants with DNA deletions are not GMOs

by Sophien Kamoun and Eric Ward

16
MAY 2012

Home About Biofortified Blog

Crop plants with DNA deletions are not GMOs

by Sophien Kamoun and Eric Ward

16
MAY 2012

- A regulatory not scientific question
- Two main regulatory frameworks for new crop varieties:
  - *process-based*
  - *product-based*

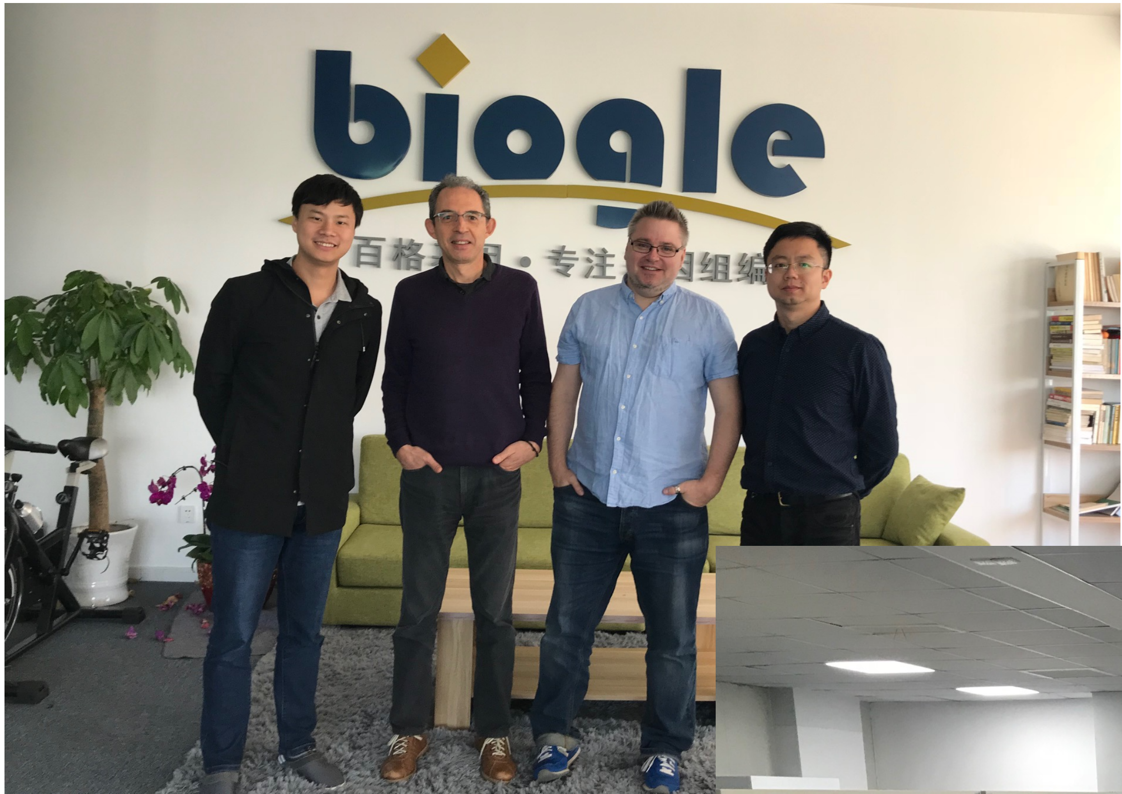
Frank Hochholdinger
@HochholdingerF

Following

The list of countries, which do not regulate targeted mutagenesis by genome editing under GMO legislation grows:
Japan 🇯🇵
USA 🇺🇸
Canada 🇨🇦
Argentina 🇦🇷
Brazil 🇧🇷
Chile 🇨🇱
Israel 🇮🇱

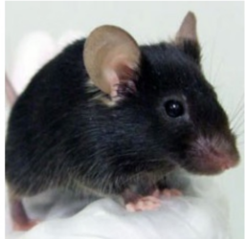
Australia 🇦🇺 is likely next.

Only the EU🇪🇺 ignores scientific consensus.


biogle


AIRTECH

-- 我们专注于基因组编辑 --



小鼠基因组编辑

- 单/多基因敲除，长片段敲除，条件性敲除
- DNA片段定点敲入、碱基替换
- 服务周期2~4个月
- 提供Founder小鼠或F1代小鼠

细胞系基因组编辑

- 常用细胞系（293T、Hela），小鼠胚胎干细胞以及iPS干细胞
- 单/多基因敲除，长片段敲除，DNA片段定点敲入、碱基替换
- 服务周期1~3个月

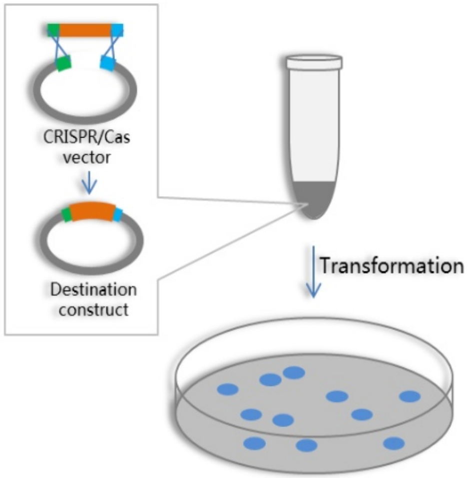
水稻基因组编辑

- 粳稻（如日本晴）、籼稻（如93-11）
- 单/多基因敲除，长片段敲除
- 服务周期2~4个月
- 提供至少3株成功突变的T0幼苗

大豆基因组编辑

- 常用品种Williams82、Jack
- 单基因敲除
- 服务周期3~9个月
- 提供至少1株成功突变的T0幼苗或3株T1幼苗

百格CRISPR/Cas载体构建试剂盒



一步载体构建，直接遗传转化，数百次的成功实验验证

--简便 无需酶切，一步载体构建，直接用于遗传转化

--快速 20^{o}C反应30~60分钟即可

--高效 1000个以上的菌落数，95%以上的阳性率，提供您所需的克隆

--可靠 数百次的植物遗传转化实验，90%以上基因敲除成功率

--广泛 涵盖拟南芥、油菜、水稻、小麦和大豆等

Genome-wide Targeted Mutagenesis in Rice Using the CRISPR/Cas9 System

Yuming Lu[1,3], Xiao Ye[1], Renming Guo[1], Jing Huang[1], Wei Wang[2], Jiuyou Tang[2], Longtao Tan[4], Jian-kang Zhu[3], Chengcai Chu[2] and Yangwen Qian[1,*]

[1]Biogle Genome Editing Center, Changzhou, Jiangsu Province 213125, China
[2]State Key Laboratory of Plant Genomics, National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
[3]Shanghai Center for Plant Stress Biology and Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai 201602, China
[4]Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
***Correspondence: Yangwen Qian (qyw@biogle.cn)**
http://dx.doi.org/10.1016/j.molp.2017.06.007

Annual Review of Plant Biology

CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture

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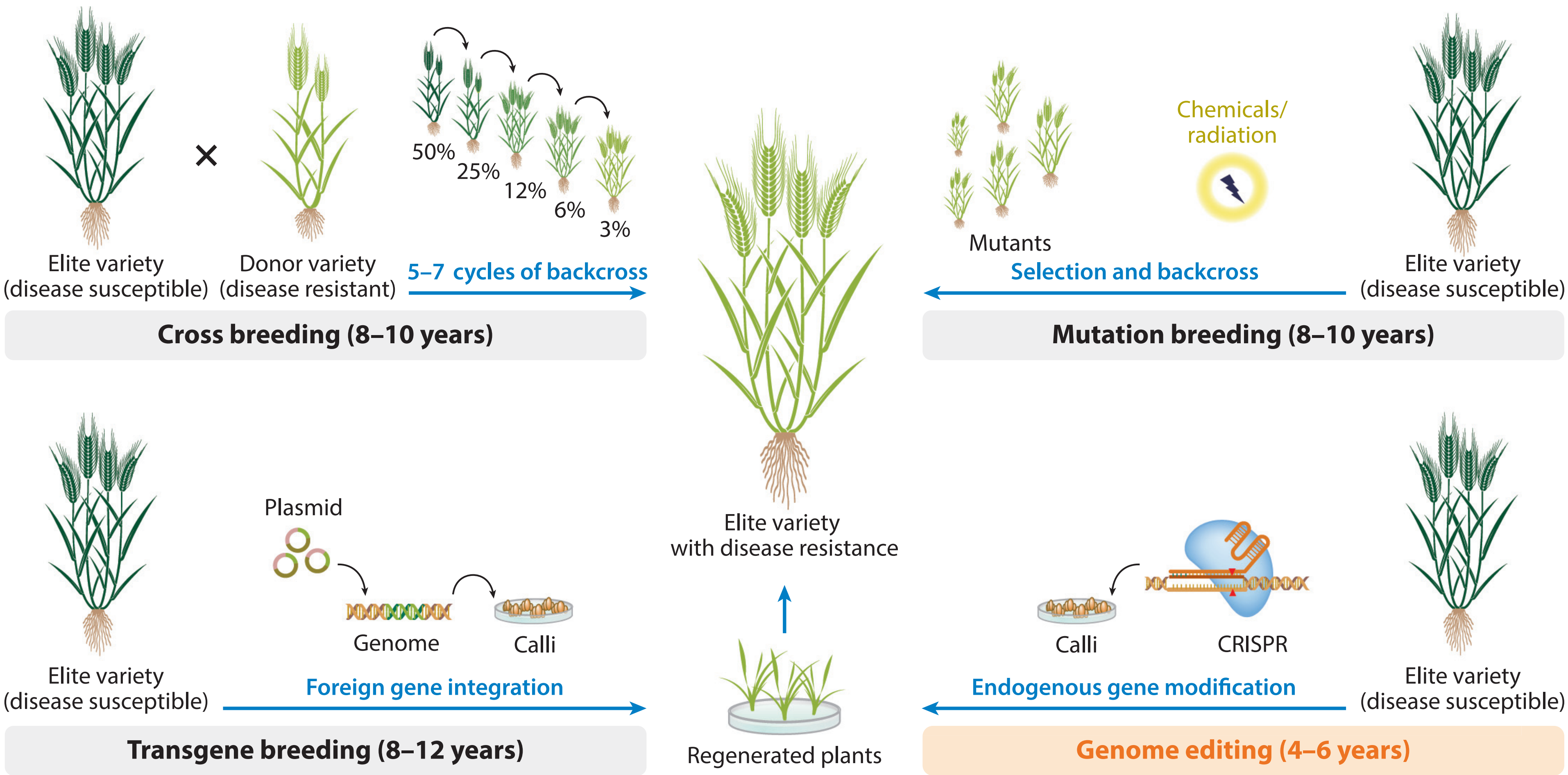


Plant Biotechnology Journal

aab Association of Applied Biologists

SEB Society for Experimental Biology

Plant Biotechnology Journal (2019) **17**, pp. 132–140

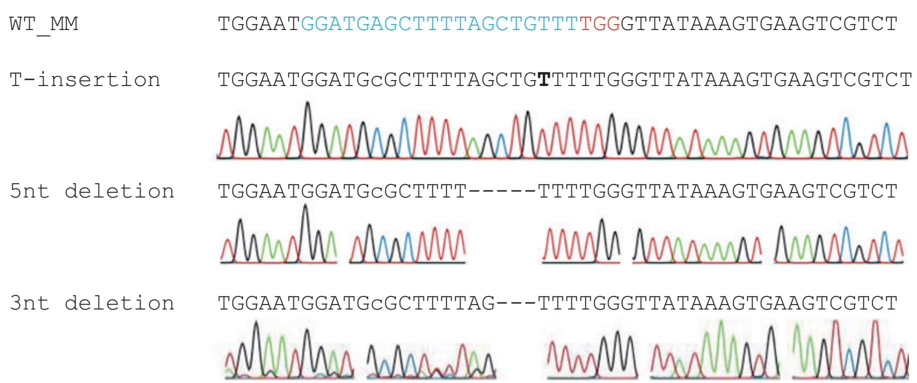
doi: 10.1111/pbi.12952

Using CRISPR/Cas9 genome editing in tomato to create a gibberellin-responsive dominant dwarf DELLA allele

Laurence Tomlinson[1], Ying Yang[1], Ryan Emenecker[2], Matthew Smoker[1], Jodie Taylor[1], Sara Perkins[1], Justine Smith[1], Dan MacLean[1], Neil E. Olszewski[2] and Jonathan D. G. Jones[1,*]

[1]*The Sainsbury Laboratory, Norwich Research Park, Norwich, UK*

[2]*Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN, USA*



Allelic series!

Plant Biotechnology Journal

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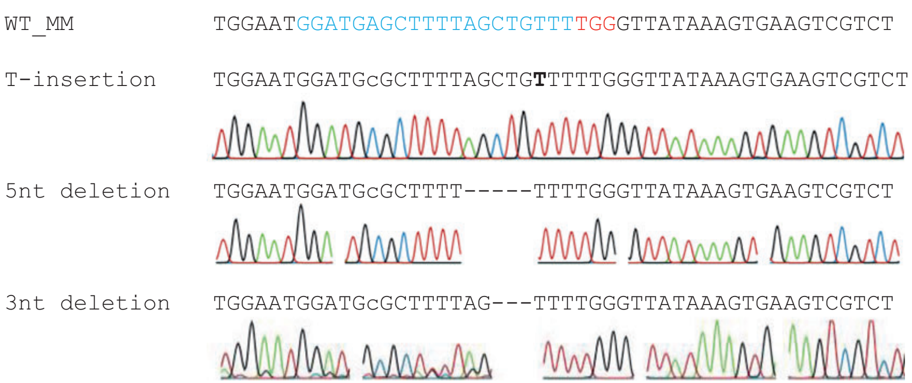

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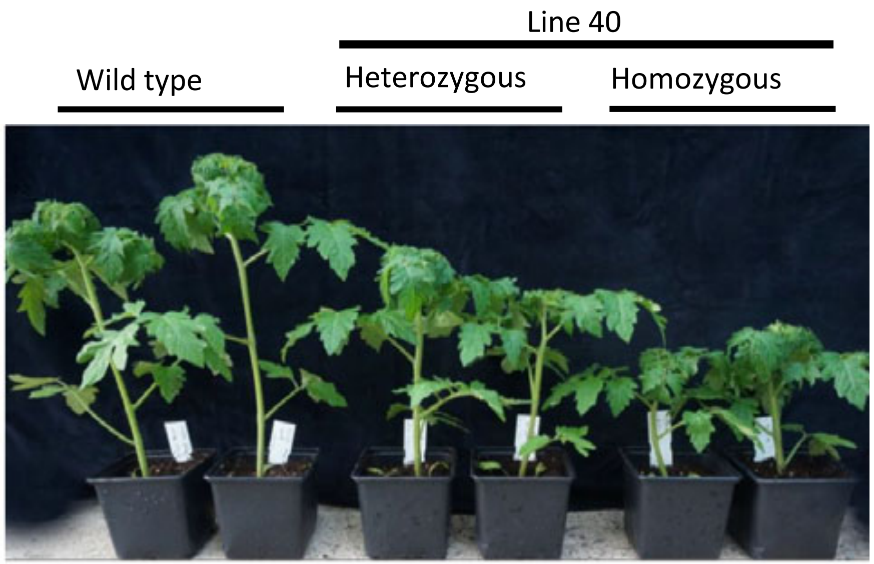
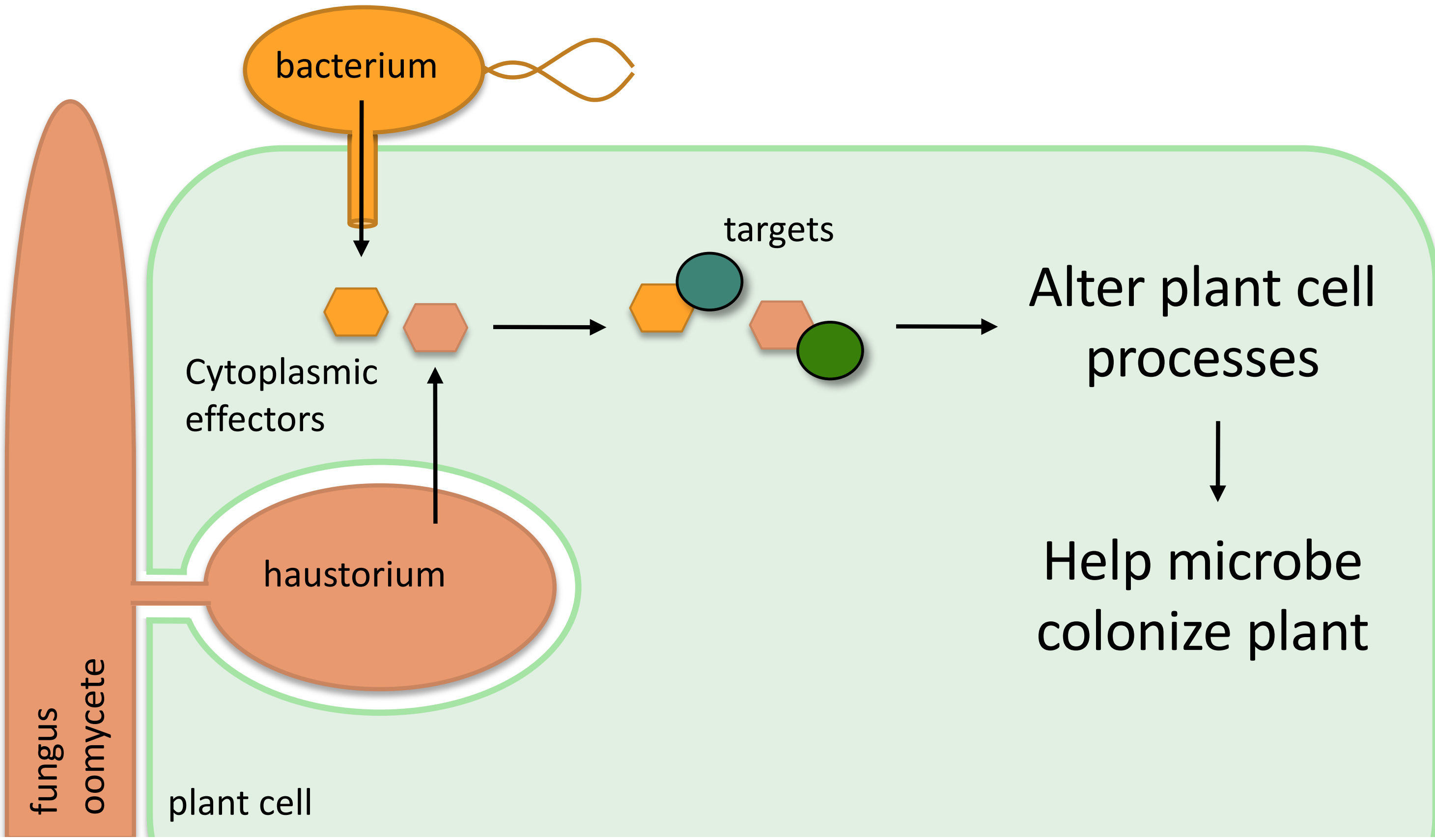


The plant targets of pathogens *facilitate* infection

Susceptibility *S* genes!



CRISPR crops—removing genes for fungal resistance

```
                     Target 1           PAM                                 PAM       Target 2
          ACATAGTAAAAGGTGTACCTGTGGTGGAGACTGGTGACCATCTTTTCTGGTTTAATCGCCCTGCCCTTGTCCTATTCTTGATTAACTTTGTACTCTTTCAGG
Plant 1   ACATAGTAAAAGGTGTACCTGTGGTGGAGACTGGTGACCATCTTTTCTGGTTTAATCGCCCTGCCCTTGTCCTATTCTTGATTAACTTTGTACTCTTTCAGG
Plant 2   ACATAGTAAAAGGTGTACCTGTGGTGGA------------------------------------------------CTTGATTAACTTTGTACTCTTTCAGG -48
Plant 8   ACATAGTAAAAGGTGTACCTGTGGTGGA------------------------------------------------CTTGATTAACTTTGTACTCTTTCAGG -48
Plant 10 |ACATAGTAAAAGGTGTACCTGTGGTGGA------------------------------------------------CTTGATTAACTTTGTACTCTTTCAGG -48
         |ACATAGTAAAAGGTGTACCTGTGGTGGA-------------------------------------------------TTGATTAACTTTGTACTCTTTCAGG -49
```

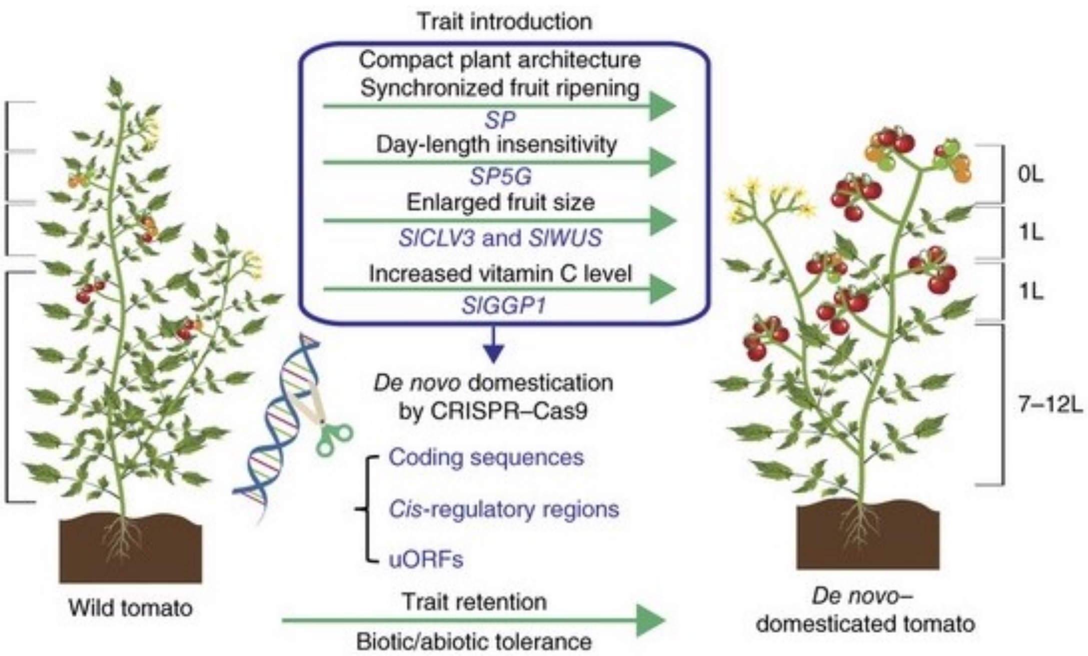
susceptible plant | resistant plant

New Tomato Ideal for Urban Gardens and Even Outer Space Created Through Genetic Editing

By COLD SPRING HARBOR LABORATORY   DECEMBER 31, 2019

De novo domestication of wild tomato using genome editing

Agustin Zsögön[1,7], Tomáš Čermák[2,6,7], Emmanuel Rezende Naves[1], Marcela Morato Notini[3], Kai H Edel[4], Stefan Weinl[4], Luciano Freschi[5], Daniel F Voytas[2], Jörg Kudla[4] & Lázaro Eustáquio Pereira Peres[3]



PLANT SCIENCES · 05 FEBRUARY 2021

Insta-crop: CRISPR enables high-speed plant domestication

A lanky species of wild rice turns compact and docile in a jiffy.

CRISPR and conservation biology

- Crop conservation biology needs a bigger toolbox to meet unprecedented challenges
  - *climate change*
  - *habitat loss*
  - *invasive pathogens and pests*
  - *etc.*

Adapted from RT Corlett Trends in Biotech 2017

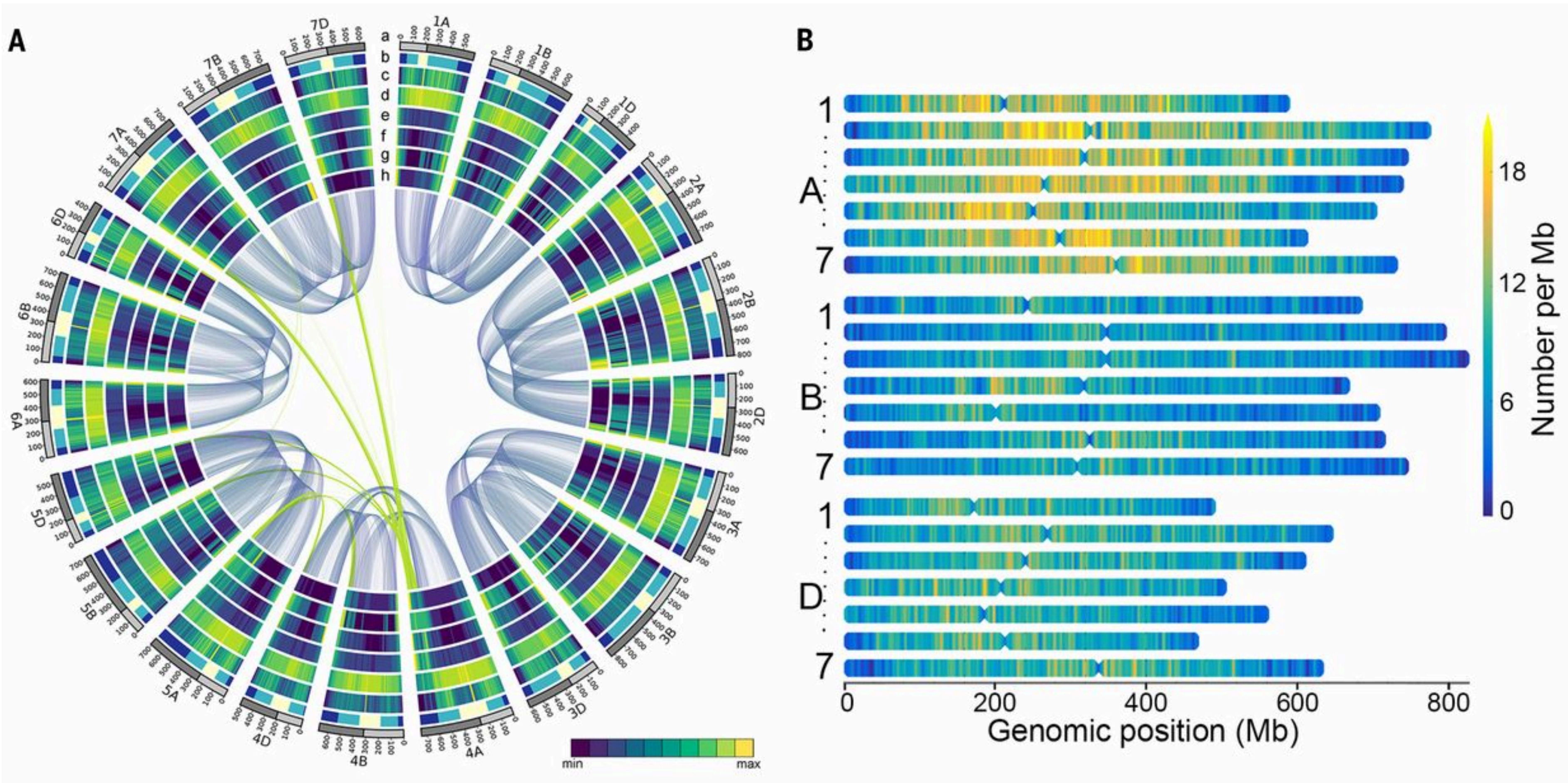
CRISPR and conservation biology

- Conservation genomics—before we conserve, we need to know what's out there
- Facilitated adaptation—introduce adaptive traits for conservation of endangered crops
- Crop de-extinction?—CRISPR can deliver it

Crop genomics—*just getting started!*

2018

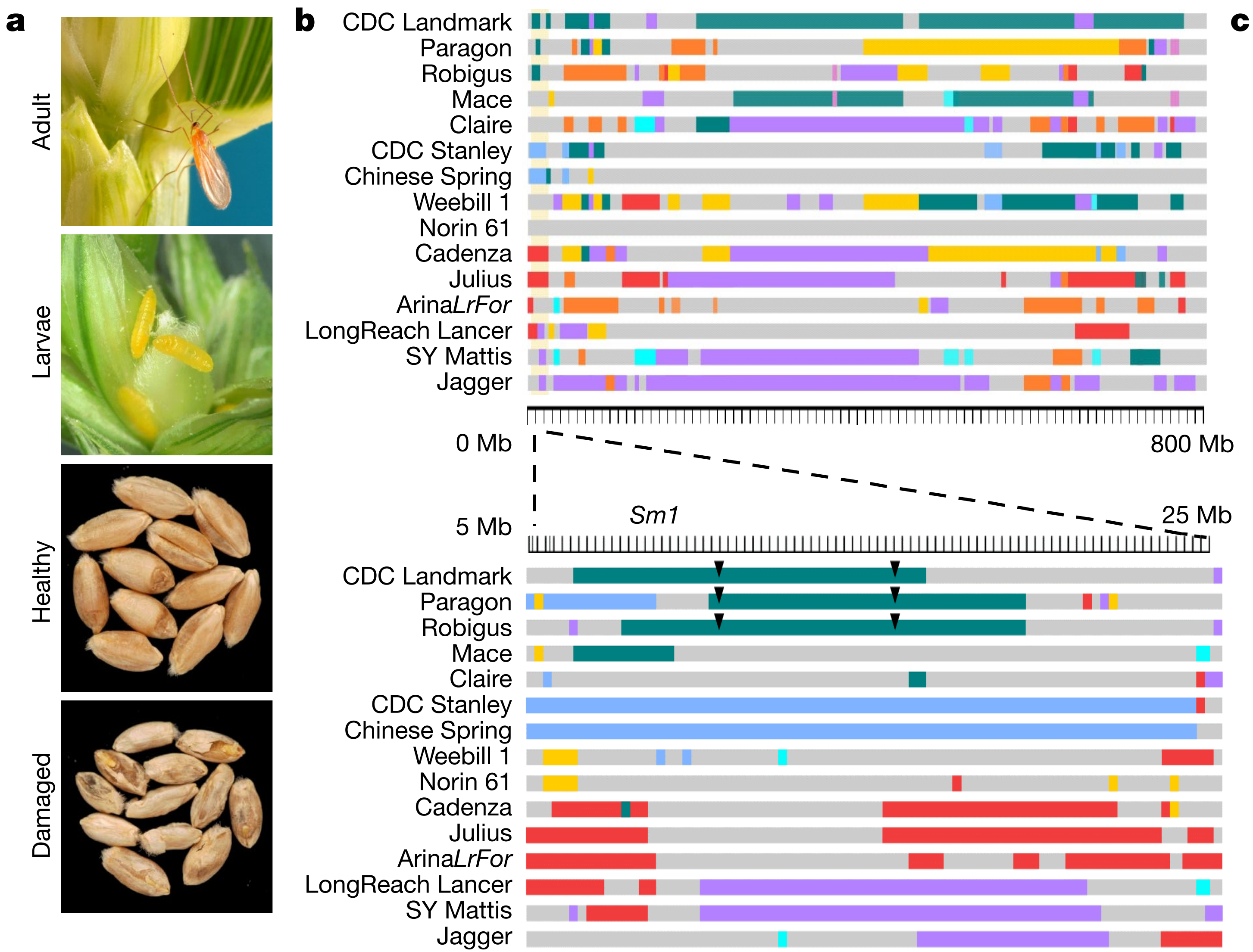
SLICING THE
wheat genome



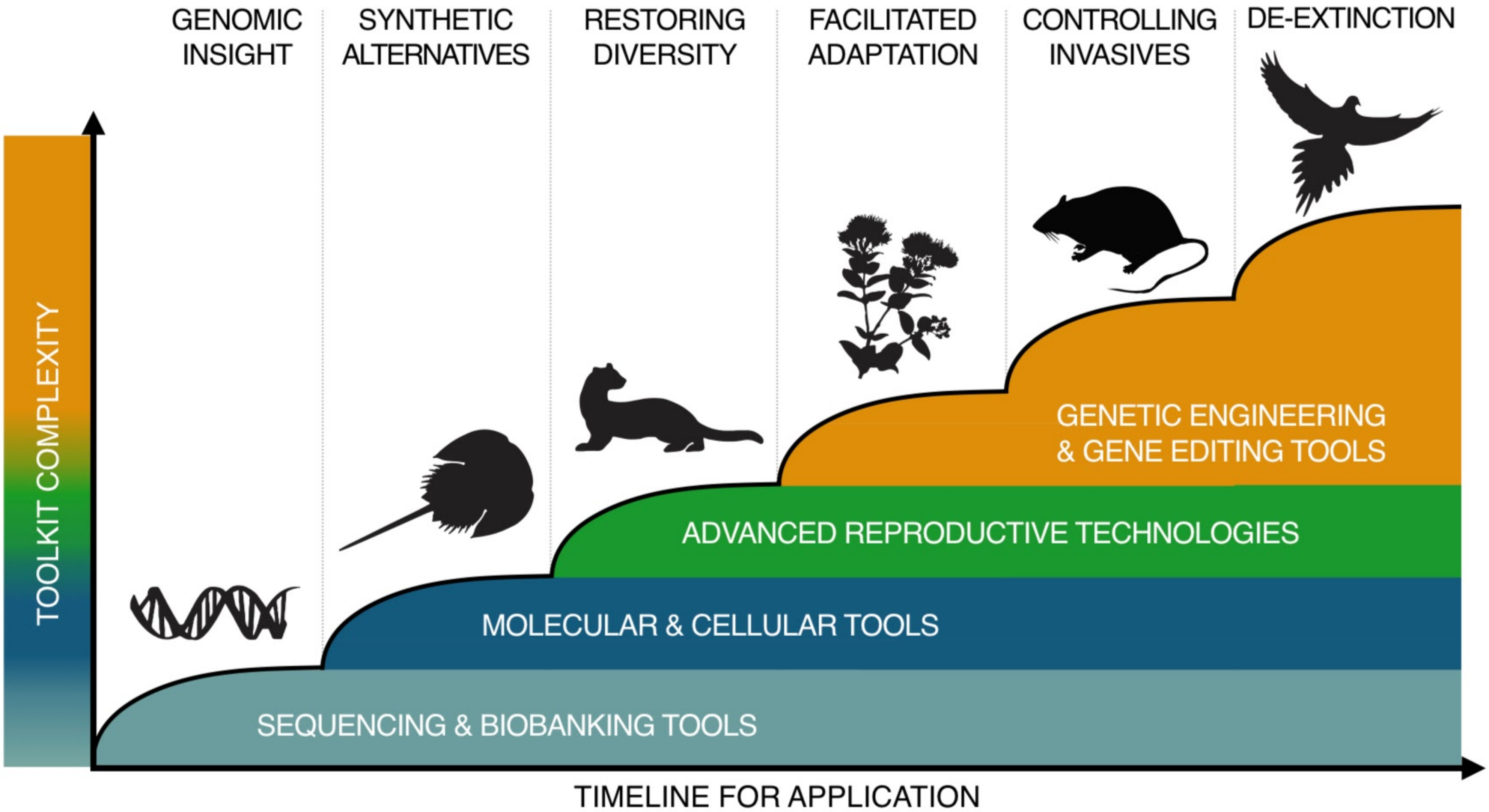
Article

Multiple wheat genomes reveal global variation in modern breeding

2020



REVIVE & RESTORE IS BRINGING BIOTECHNOLOGIES TO CONSERVATION



revive&restore

WHAT WE DO PROJECTS FUNDING OPPORTUNITIES EVENTS BLOG ABOUT US DONATE

Plant genome editing is in its infancy…

Kamoun Lab @ TSL

Sophien Kamoun
@KamounLab

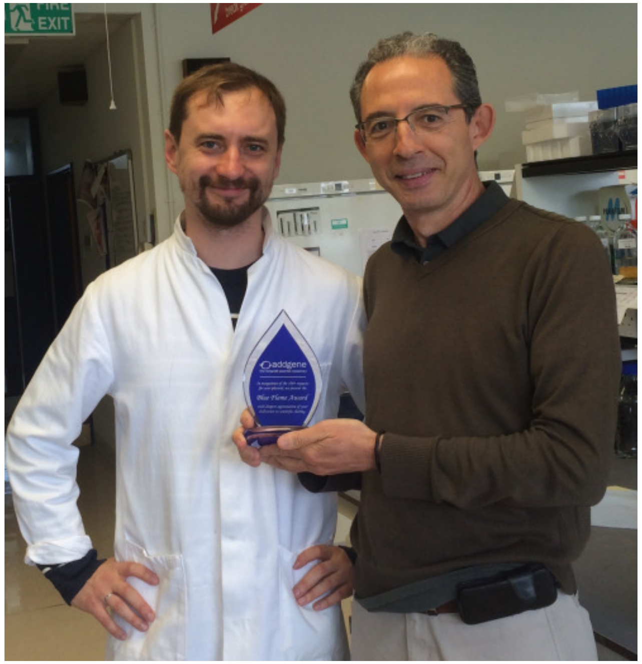
"I didn't come here to tell you how this is going to end. I came here to tell you how it's going to begin." Neo 'The Matrix'

Acknowledgements

Khaoula Belhaj

Angela Chaparro Garcia

Joe Win



Vladimir Nekrasov

Diana Gomez

Adeline Harant

Thorsten Langner

Great Resource: Plant Genome Editing Database /via Greg Martin @BTIscience @NSF

Home

Resource: Plant Genome Editing Database (2018) | Plants and Microbes

scoop.it

29/10/2018, 19:13