

CRISPR Technology

Gene Editing Possibilities

Mint Industry Research Council National Meeting
January 2017 / San Diego, CA

Steve Strauss

Oregon State University

Steve.Strauss@OregonState.Edu



Breakthrough of the Year, 2015

Science

AAAS

2015

BRE
of t

JULY 4, 2015

TIME

The Gene Machine

What the CRISPR experiments mean for humanity *By Alice Park*

time.com

Agenda

1. Genetic engineering defined
2. Gene editing defined
3. Examples and applications
4. Our work with CRISPR
5. Society, regulation, markets

Coauthor Estefania Elorriaga



PhD student, Molecular
and Cellular Biology, OSU

Research on gene editing
in poplar and eucalypts

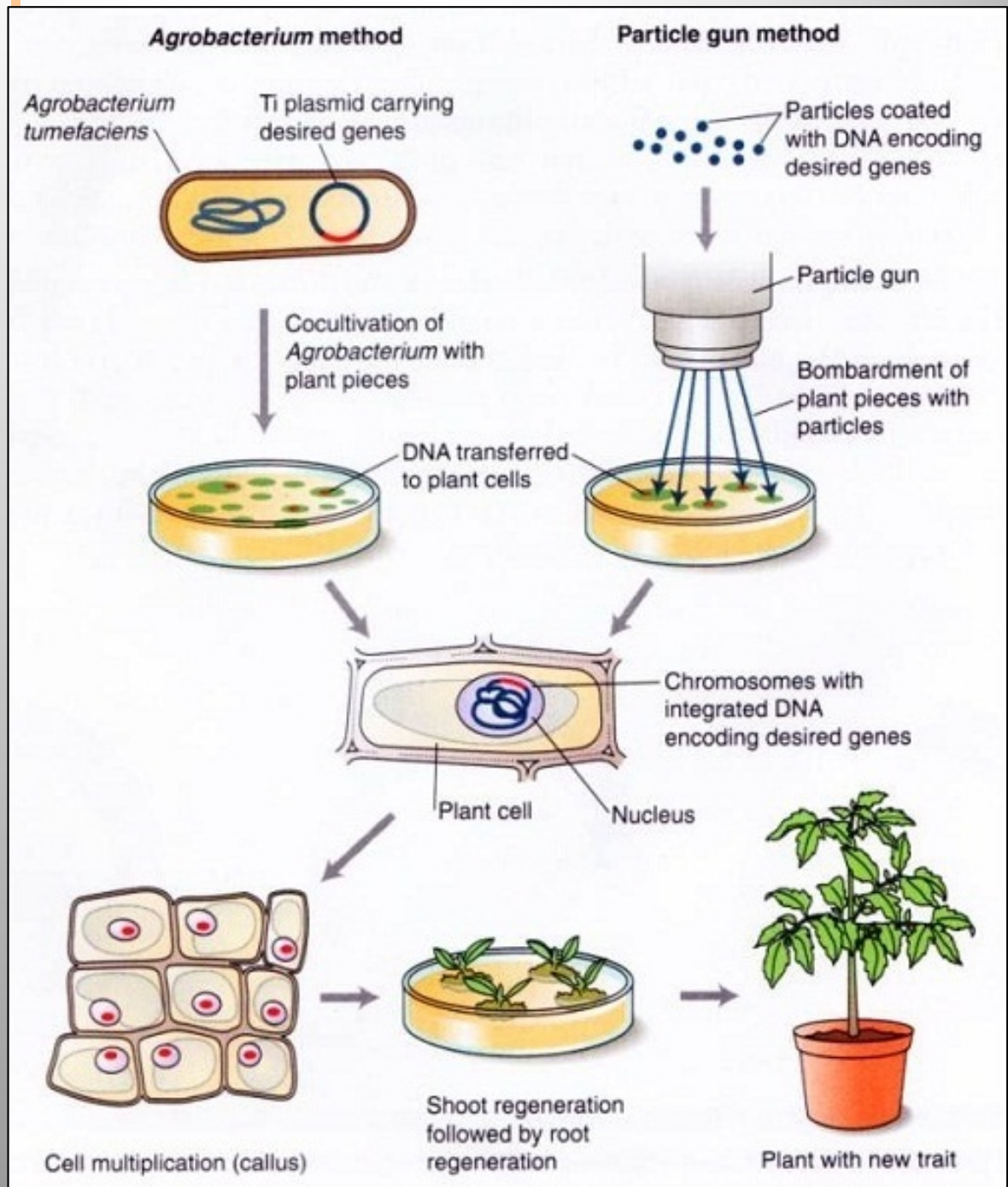
What is genetic engineering (GE)

- Direct modification of DNA
 - Vs. indirect modification in breeding and genomic selection
- Asexually modified in somatic cells
 - Then regenerated into whole organisms, usually starting in Petri dishes



Steps to create a GE plant

- *Agrobacterium*-mediated transformation
- Biolistics or gene gun



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A big deal?

- Ability to modify native genes efficiently
- The theoretical becomes practical



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ScienceDirect

Current Opinion in
Biotechnology

Editing plant genomes with CRISPR/Cas9

Khaoula Belhaj¹, Angela Chaparro-Garcia¹, Sophien Kamoun,
Nicola J Patron and Vladimir Nekrasov



CRISPR/Cas9 is a rapidly developing genome editing technology that has been successfully applied in many organisms, including model and crop plants. Cas9, an RNA-guided DNA endonuclease, can be targeted to specific genomic sequences by engineering a separately encoded guide RNA with which it forms a complex. As only a short RNA sequence must be synthesized to confer recognition of a new

nucleases, the repair may be imperfect. HDR, however, uses a template for repair and therefore repairs are likely to be perfect. In a natural situation the sister chromatid would be the template for repair, however templates to recode a target locus or to introduce a new element between flanking regions of homology can be delivered with an SSN [2]. In mammalian cells, DSBs were shown

“CRISPR/Cas9 is a game-changing technology that is poised to revolutionize basic research and plant breeding.”

Gene editing described



- Technique that allows specific changes to the genome
- Employs methods of genetic engineering but generally does not leave the editing agent in the genome
 - Editing agent enters cell but does not become part of genome
 - Editing agent sexually segregated away (progeny chosen with the edit, but not the editing agent)
 - Or agent somatically excised after editing

CRISPR gene editing system can be used for multiple purposes

- Mutations to destroy gene function
- Directed changes to sequence to change function
 - Proteins, RNAs, regulatory regions
- Gene or chromosome scale rearrangements (inversions, translocations)
- Ability to readily multiplex and mutate numerous genes at once
- Gene insertions directed at specific places
- Very low off-target rate in plants
- Conversion of alleles in successive generations (gene drive) – a useful means for control of serious diseases, pests, invasive exotic species?

Multiplex CRISPR:62 genes targeted

Science

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Science Science Advances Science Immunology Science Robotics Science Signaling Science Translational Medicine

SHARE REPORT

Genome-wide inactivation of porcine endogenous retroviruses (PERVs)

Luhan Yang^{1,2,3,*†}, Marc Güell^{1,2,3,†}, Dong Niu^{1,4,†}, Haydy George^{1,†}, Emal Leshah¹, Dennis Grishin¹, John Aach¹, Ellen Shrock¹, Weihong Xu⁶, Jürgen Poci¹, Rebeca Cortazio¹, Robert A. Wilkinson⁵, Jay A. Fishman⁵, George Church^{1,2,3,*}

+ Author Affiliations

*Corresponding author. E-mail: gchurch@genetics.med.harvard.edu (G.C.); luhan.yang@eGenesisbio.com (L.Y.)

† These authors contributed equally to this work.

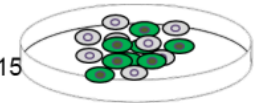
Science 27 Nov 2015;
Vol. 350, Issue 6264, pp. 1101-1104
DOI: 10.1126/science.aad1191

Virally cleansing the pig genome

Transplants from pigs could be a solution to a shortage of human organs for transplantation. Unfortunately, porcine endogenous retroviruses (PERVs) are rife in pigs and can be transmitted to humans, risking disease. L. Yang *et al.* integrated CRISPR-Cas into the pig cell genome, where continuous induction of the Cas9 editing enzyme resulted in the mutation of every single PERV reverse transcriptase gene. This prevented replication of all copies of PERV, viral infection, and transmission to human cells.

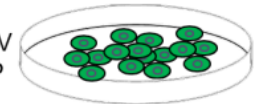
Science, this issue p. 1101

Co-culture
HEK-293-GFP & PK15



Sort /
Puro
selection

Detection of PERV
In HEK-293-GFP



Application to mint

- CRISPR most useful in crop systems where breeding is difficult, clonal varieties of high value, there are traits of value under simple genetic control (few genes)
- Mint breeding
 - Few elite clones
 - Complex ploidy (multiple chromosome sets)
 - Fertility very low
 - Undirected mutagenesis methods important
- Possible mint targets
 - Oil yield and quality
 - Disease resistance (Verticillium, others)
 - Any trait where the genic basis is known – growing with science
 - Genome sequence, QTL, and gene expression data growing
 - Molecular biology of terpenoid pathways – novel high value products?

Polyploid gene editing is effective


[Plant Cell Reports](#)

January 2017, Volume 36, [Issue 1](#), pp 117–128

Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts

[Authors](#)

[Authors and affiliations](#)

Mariette Andersson, Helle Turesson, Alessandro Nicolia, Ann-Sofie Fält, Mathias Samuelsson, Per Hofvander 

[Open Access](#) | [Original Article](#)

First Online: **03 October 2016**

DOI: [10.1007/s00299-016-2062-3](https://doi.org/10.1007/s00299-016-2062-3)

Cite this article as:

Andersson, M., Turesson, H., Nicolia, A. et al.

Plant Cell Rep (2017) 36: 117.

doi:[10.1007/s00299-016-2062-3](https://doi.org/10.1007/s00299-016-2062-3)

7

Shares

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Abstract

Key message

Altered starch quality with full knockout of *GBSS* gene function in potato was achieved using CRISPR-Cas9 technology, through transient transfection and regeneration from isolated protoplasts.

CRISPR/Cas systems are the dominant form of gene editing

- CRISPR stands for Clustered, Regularly Interspaced, Short Palindromic Repeats
- The CRISPR/Cas system is an adaptive defense system in prokaryotes to fight against alien nucleic acids

Defense system in nature

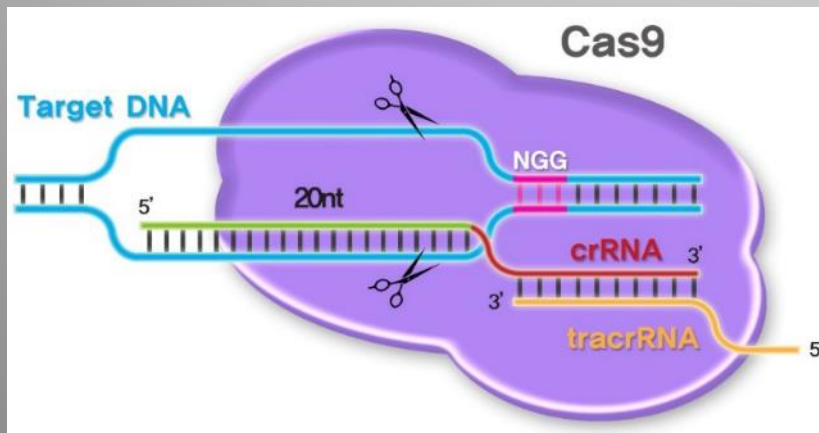
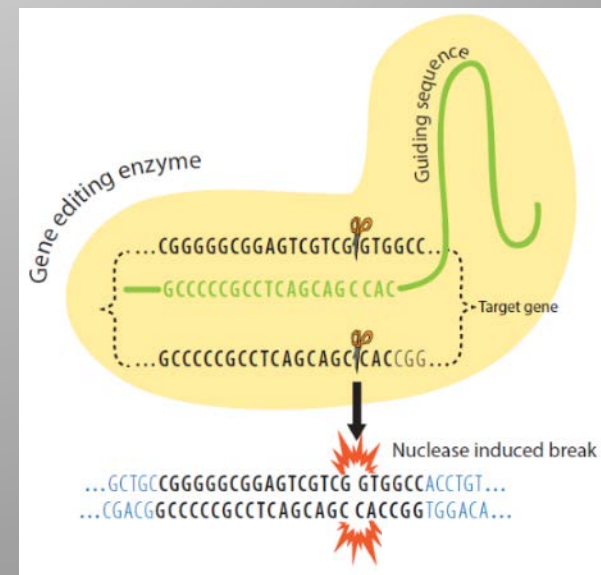


Image credit: <http://pnabio.com/products/RGEN.htm>

Synthetic nuclease system



A global scientific achievement

20 years of CRISPR

“The story starts in the Mediterranean port of Santa Pola on Spain’s Costa Blanca, ...in a laboratory working on *Haloferax mediterranei*, an archaeal microbe with extreme salt tolerance...the salt concentration of the growth medium appeared to affect the way in which restriction enzymes cut the microbe’s genome...the [derived] fragments...did not resemble any family of repeats known in microbes..”



- | | | | | | | | | |
|---|------|---|---|------|---|----|------|---|
| 1 | 1993 | Discovery of CRISPR | 4 | 2008 | Programming CRISPR | 8 | 2011 | Reconstituting CRISPR in a distant organism |
| 2 | 2003 | CRISPR is an adaptive immune system | 5 | 2008 | CRISPR targets DNA | 9 | 2012 | Studying CRISPR in vitro |
| 3 | 2006 | Experimental evidence that CRISPR confers adaptive immunity | 6 | 2010 | Cas9 is guided by crRNAs and creates double-stranded breaks | 10 | 2012 | Genome editing in mammalian cells |
| | | | 7 | 2010 | Discovery of tracrRNA | | | |

Lessons from CRISPR discovery

(Lander 2006, Cell)

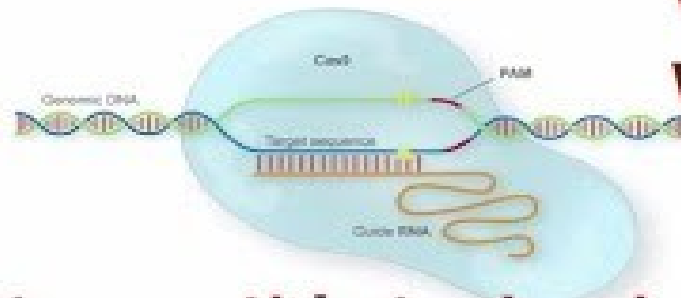
- Breakthroughs often emerge from unpredictable origins
 - Motivations included curiosity, and military and industrial applications
- Growing “hypothesis-free” omic discovery
- Best science work early in career – often pre-30
- Seminal work not from eminent research centers
- Leading journals rejected all the early papers
- Science is a slow, global enterprise, with multiple authors and institutions contributing

Science journalist Carl Zimmer explains CRISPR DNA editing in 90 seconds



A video with a more technical look at CRISPR

CRISPR | Cas9



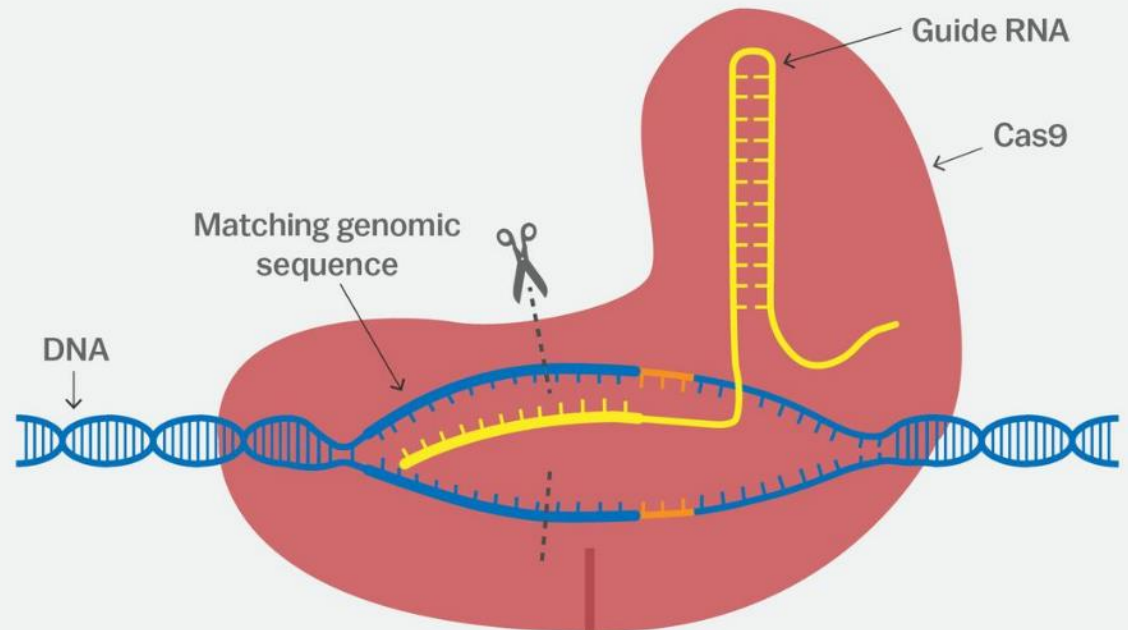
What is CRISPR?
What is Cas9?

How to use this technology?
Learn from this 4-min Video

Sandman CRISPR !



Summary of CRISPR Cas- mechanism

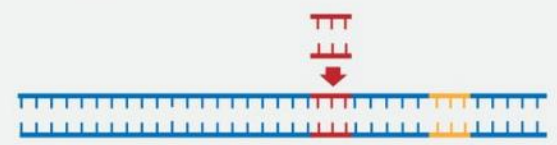


DELETING A GENE

INSERTING A GENE



Gene is disrupted



Gene has a new sequence

Two major types of edits

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RNA

interference

(RNAi)

A natural mechanism of gene suppression.

Many products on the market. Gene editing products do similar things without the transgene present



The Nobel Prize in Physiology or Medicine 2006
Andrew Z. Fire, Craig C. Mello

Share this:     28 

The Nobel Prize in Physiology or Medicine 2006



Photo: L. Cicero
Andrew Z. Fire
Prize share: 1/2



Photo: J. Mottern
Craig C. Mello
Prize share: 1/2

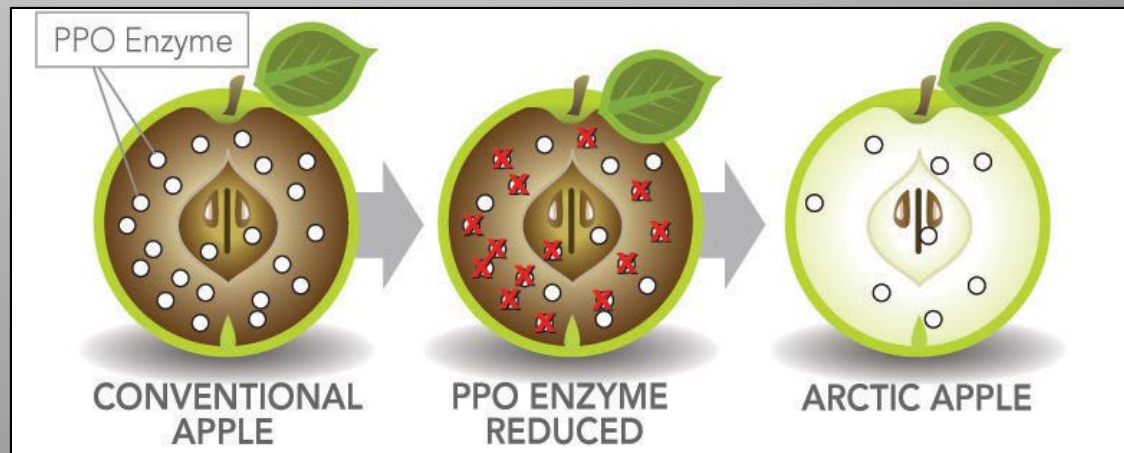
The Nobel Prize in Physiology or Medicine 2006 was awarded jointly to Andrew Z. Fire and Craig C. Mello "for their discovery of RNA interference - gene silencing by double-stranded RNA"

Non-browning “Arctic Apple”

RNAi suppression of native polyphenol oxidase gene expression



Courtesy of Jennifer Armen,
Okanagan Specialty Fruits,
Canada



CRISPR non-browning mushroom with mutated polyphenol oxidase (PPO) gene

nature International weekly journal of science

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Archive > Volume 532 > Issue 7599 > News > Article

NATURE | NEWS 🔗 ✉ 🖨

Gene-edited CRISPR mushroom escapes US regulation


A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight.

Emily Waltz

14 April 2016




Non-bruising PPO- and invertase-mutant potatoes produced by gene editing



Crop Biotech Update

A weekly summary of world developments in agri-biotech for developing countries, produced by the Global Knowledge Center on Crop Biotechnology, International Service for the Acquisition of Agri-biotech Applications SEAsia Center (ISAAA).

www.isaaa.org/kc/cropbiotechupdate/ www.isaaa.org 

Articles in the [November 3, 2016 Issue of Crop Biotech Update](#)

NON-BRUIISING GE POTATO CLEARED FOR SALE BY USDA




The [GE potato](#) that withstands bruising and browning has been cleared for sale by the U.S. Department of Agriculture. According to USDA, the GE potato is not considered as "regulated article" under federal law because it does not contain [genes](#) from plant pests.

The GE potato was developed by Calyxt, Inc. by introducing a TALEN reagent into potato



Improved Quality Potato

gene glycol mediated transformation followed by temporary protoplast to achieve PPO gene knockout and regeneration of protoplast plants. Thus, there is no foreign genetic material inserted into



Plant Biotechnology Journal (2016) 14, pp. 169–176 doi: 10.1111/pbi.12370

Improving cold storage and processing traits in potato through targeted gene knockout

Benjamin M. Clasen¹, Thomas J. Stoddard¹, Song Luo¹, Zachary L. Demorest¹, Jin Li¹, Frederic Cedrone², Redeat Tibebe¹, Shawn Davison¹, Erin E. Ray¹, Aurelie Daulhac¹, Andrew Coffman¹, Ann Yabandith¹, Adam Retterath¹, William Haun¹, Nicholas J. Baltes¹, Luc Mathis¹, Daniel F. Voytas¹ and Feng Zhang^{1,*}

¹Collectis plant sciences Inc., New Brighton, MN, USA
²Collectis SA, Paris, France

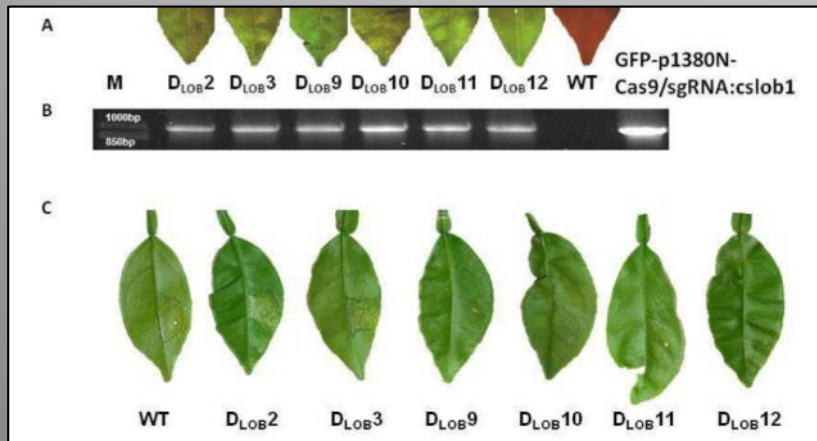
Pioneer's CRISPR-edited *waxy* corn of high commercial value, unregulated



© Dinodia Photos / Alamy Stock Photo

DuPont Pioneer's high amylopectin corn is the first CRISPR-edited plant likely to bypass USDA oversight.

CRISPR- modified grapefruit resistant to citrus canker



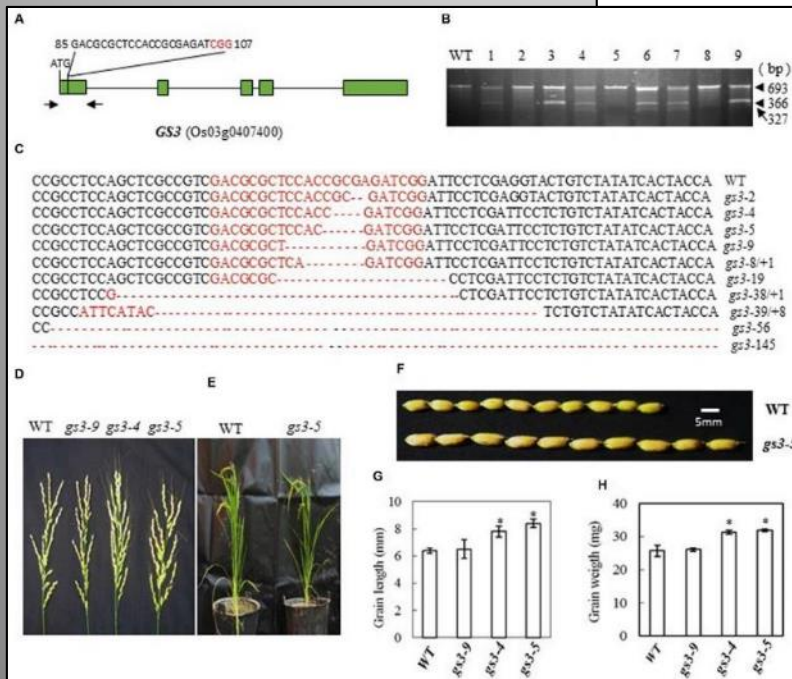
- Other examples:
 - Blight resistant rice (Zhou et al., 2014, Nucl. Acids Res.)
 - Fungus-resistant wheat (Wang et al., 2014, Nat Biotech)
 - Virus-resistant cucumber (Chandrasekaran et al., 2016, Molecular Plant Pathology)

Mutated yield-related genes in wheat leads to larger and more numerous grains

Reassessment of the Four Yield-related Genes *Gn1a*, *DEP1*, *GS3*, and *IPA1* in Rice Using a CRISPR/Cas9 System

Meiru Li^{1,2†}, Xiaoxia Li^{3†}, Zejiao Zhou³, Pingzhi Wu^{1,2}, Maichun Fang^{1,2}, Xiaoping Pan^{1,2}, Qiupeng Lin³, Wanbin Luo³, Guojiang Wu^{1,2*} and Hongqing Li^{3*}

¹ Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China, ² Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China, ³ Guangdong Provincial Key Lab of Biotechnology for Plant Development, South China Normal University, Guangzhou, China



“...The T2 generation of the *gn1a*, *dep1*, and *gs3* mutants featured enhanced grain number, dense erect panicles, and larger grain size, respectively....” Li et al. (2016)

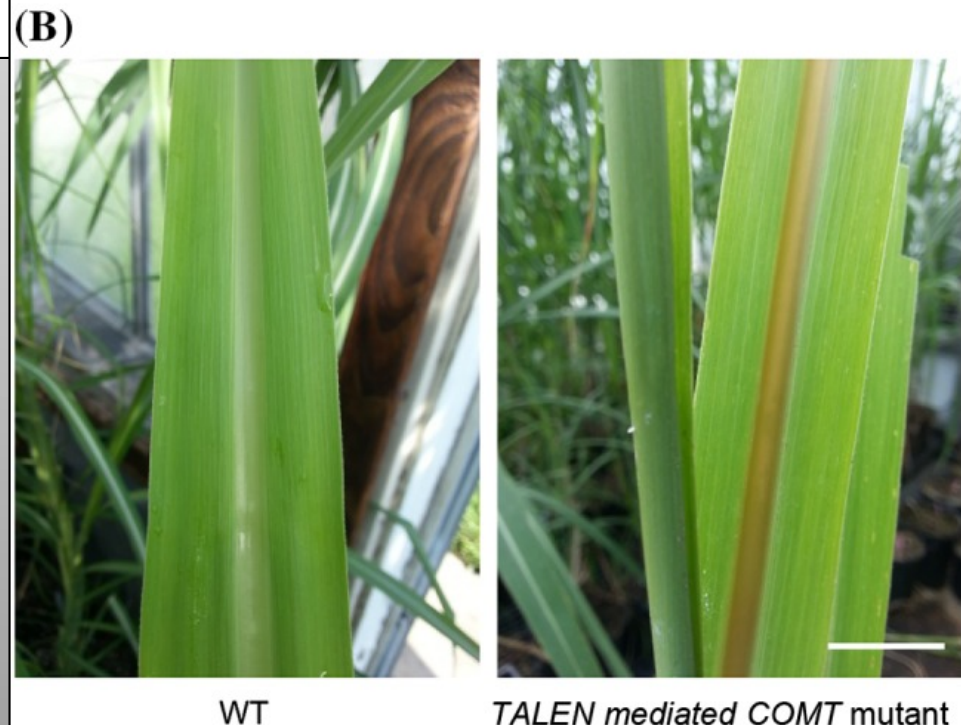
Sugarcane, a polyploid, with lower lignin for use as biofuel

Plant Mol Biol (2016) 92:131–142
DOI 10.1007/s11103-016-0499-y



TALEN mediated targeted mutagenesis of the *caffeic acid O-methyltransferase* in highly polyploid sugarcane improves cell wall composition for production of bioethanol

Je Hyeong Jung^{1,4} · Fredy Altpeter^{1,2,3}



- Brown coloration seen in plants with high lignin reduction (22 to 32% reduction)
- Altpeter, 2016, Plant Molecular Biology

Other applications

- Soybean seeds with improved fatty acid content/profile (Haun et al. 2014, Plant Biotechnol Journal)
- Tobacco with improved glycosylation profiles for safer/faster production of pharmaceutical proteins (Li et al., 2016, Plant Biotechnol Journal 14)

Recombinetics creates hornless cattle

Open Season Is Seen in Gene Editing of Animals

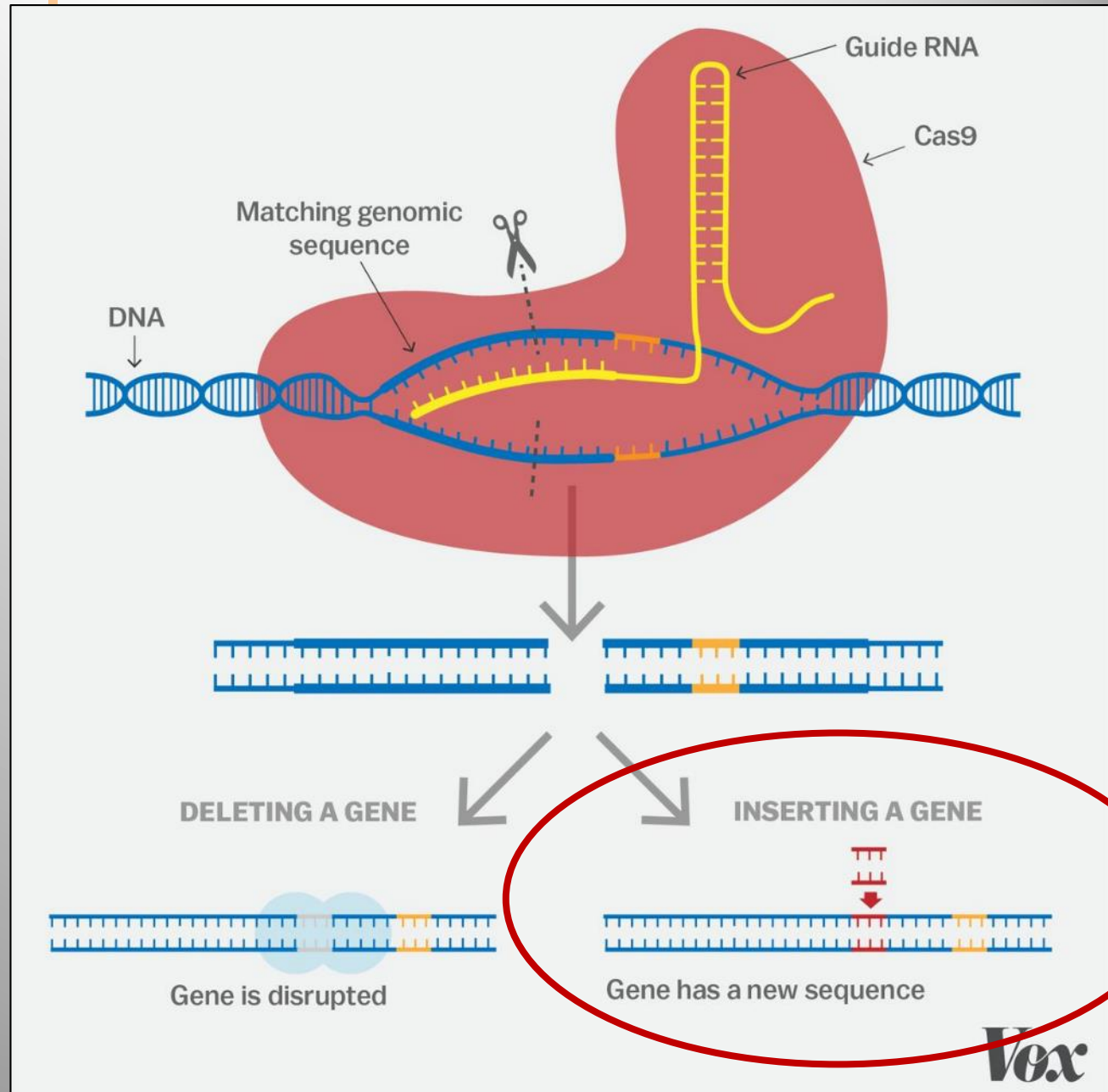
By AMY HARMON NOV. 26, 2015



A calf, left, approximately the same age as the first two genetically modified calves to have their DNA edited so that they do not grow horns, right. Jenn Ackerman for *The New York Times*

The New York Times

Directed gene modification



Homology-directed repair leads to herbicide-resistant crops

- Chlorsulfuron-resistant maize (Svitashev et al., 2015, Plant Physiology)
- Chlorsulfuron-resistant potato (Butler et al., 2016, Front. Plant Science)
- Chlorsulfuron-resistant soybean (Li et al., 2015, Plant Physiology)
- Chlorsulfuron-resistant rice (Sun et al., 2016, Molecular Plant)
- Glyphosate-resistant rice (Li et al., 2016, Nature Plants)



Promoter replacement using CRISPR increased grain yield in maize

Plant Biotechnology Journal
Open Access


aab Association of Applied Biologists
SEB Society for Experimental Biology

Explore this journal >

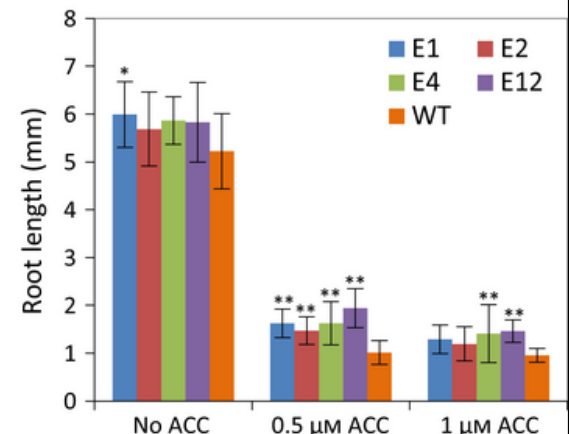
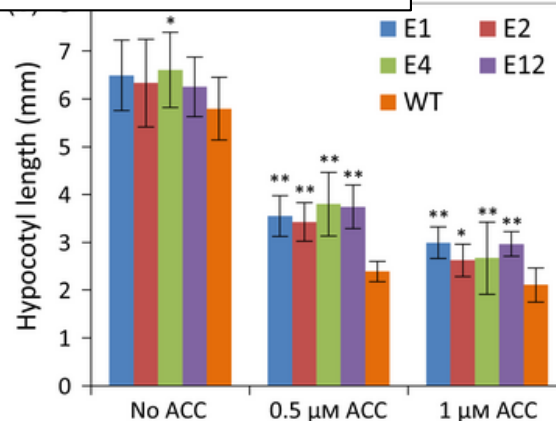
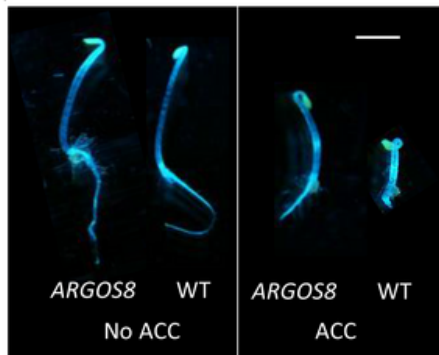
Open Access  Creative Commons

Research Article

ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions

Jinrui Shi , Huirong Gao, Hongyu Wang, H. Renee Lafitte, Rayeann L. Archibald, Meizhu Yang, Salim M. Hakimi, Hua Mo, Jeffrey E. Habben

Promoter replacement of the endogenous *ARGOS8* gene leads to increased grain yield during flowering stress and no yield loss during well-watered condition



A strong promoter, modified by gene editing, increased anthocyanin biosynthesis in tomato



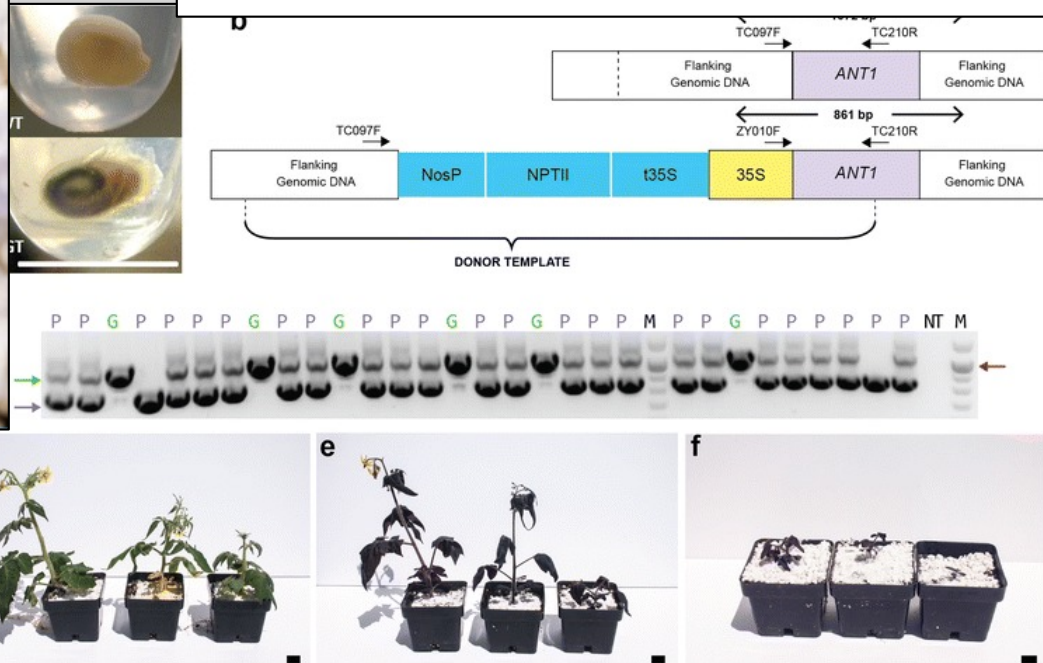
Čermák et al. *Genome Biology*
DOI 10.1186/s13059-015-0796-9

Genome **Biology**

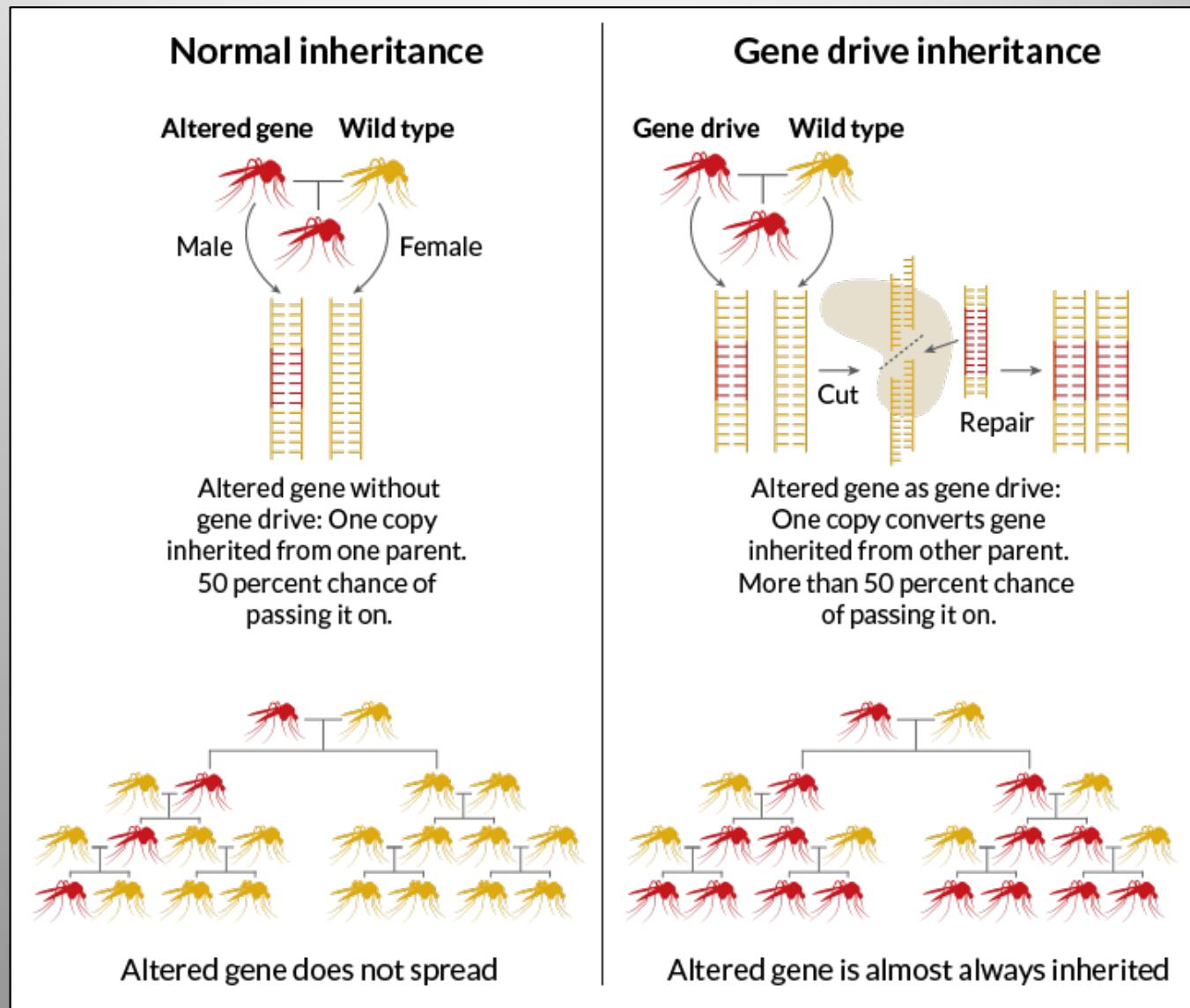
RESEARCH Open Access

High-frequency, precise modification of the tomato genome

Tomáš Čermák¹, Nicholas J. Baltes¹, Radim Čegan², Yong Zhang³ and Daniel F. Voytas^{1*}



Gene drives for suppression of crop pests?



THE NEW YORKER

ANNALS OF SCIENCE JANUARY 2, 2017 ISSUE

REWRITING THE CODE OF LIFE

Through DNA editing, researchers hope to alter the genetic destiny of species and eliminate diseases.

By Michael Specter



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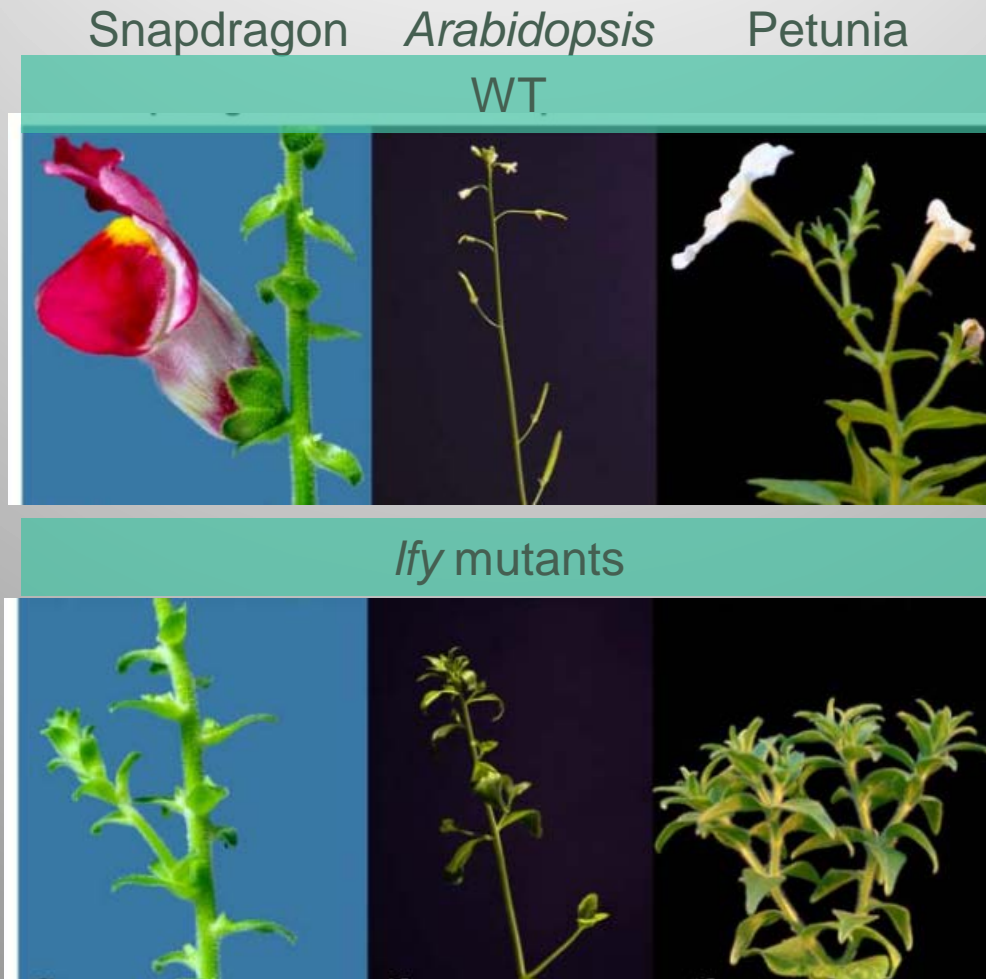
CRISPR-Cas9 targeting of floral genes for genetic containment

- **Goal:** To develop robust male and female containment technologies for vegetatively propagated forest trees
- **Why:** Regulatory, market, and public acceptance with exotic and native trees can be costly or impossible – even for field research
- **Advantage of gene editing:** Expected to be more predictable and stable than alternative genetic containment methods that depend on modified gene expression
- **High efficiency:** Biallelic knock-outs needed in one or more genes

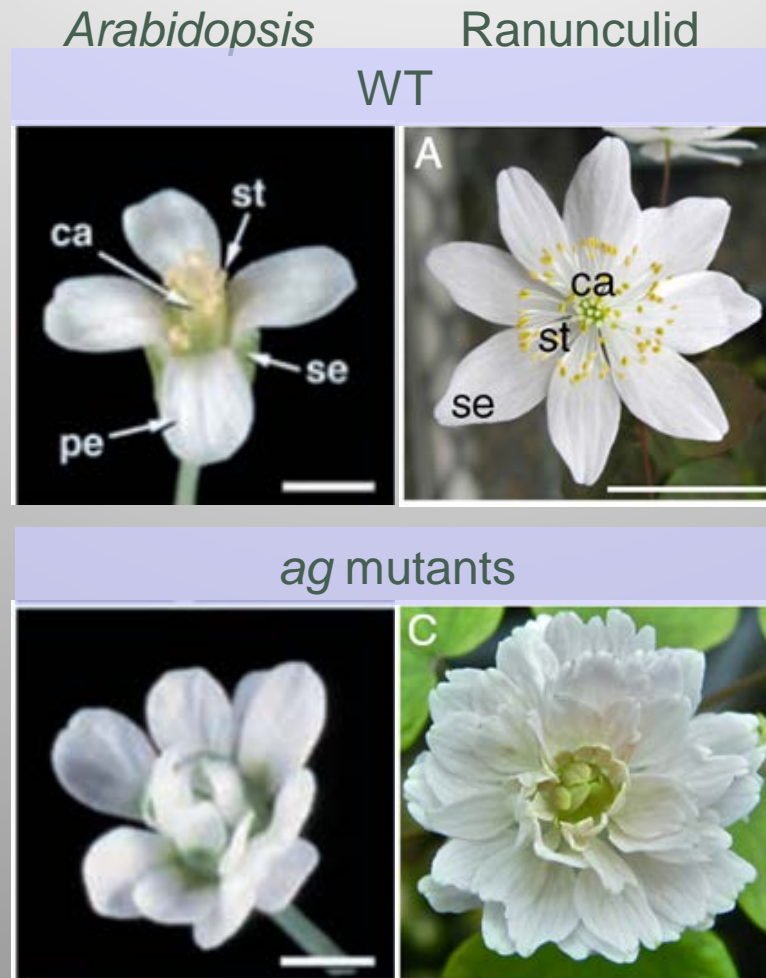
Target genes for bisexual sterility

- *LEAFY* – floral meristem prior to organ differentiation
- *AGAMOUS* – Male and female organ development and floral determinacy

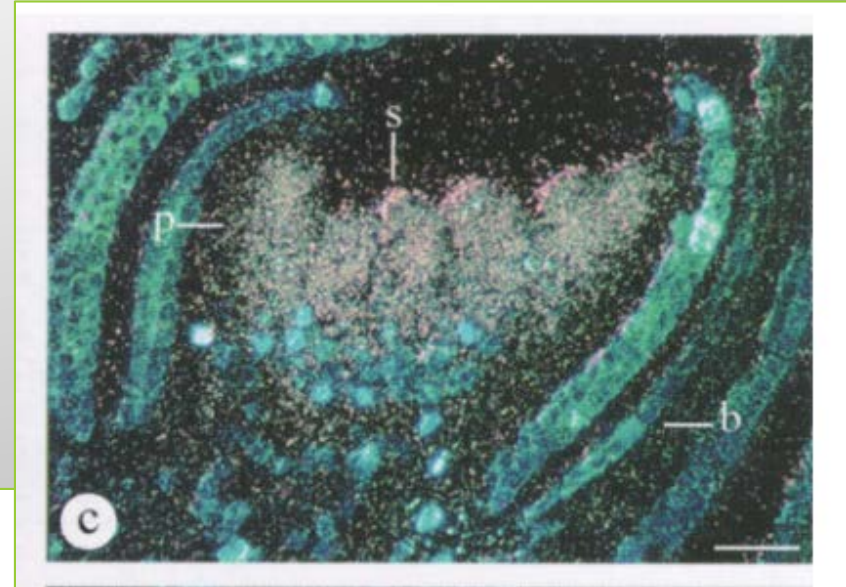
Strong *lfy* mutants appear to have no flowers



Flowers in strong *ag* mutants are missing both stamens and carpels



LEAFY and AGAMOUS homologs in poplar studied in prior work



Plant Molecular Biology 44: 619-634, 2000. © 2000 Kluwer Academic Publishers. Printed in the Netherlands.

619

Structure and expression of duplicate AGAMOUS orthologues in poplar

Amy M. Brunner, William H. Rottmann¹, Lorraine A. Sheppard², Konstantin Krutovskii, Stephen P. DiFazio, Stefano Leonardi³ and Steven H. Strauss*

Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA (*author for correspondence; e-mail: strauss@fst.orst.edu); present addresses: 1 Westvaco Forest Science and Technology, PO. Box 1950, Summerville, SC 29484, USA; 2 Institute of Forest Genetics, USDA Forest Service c/o Department of Environmental Horticulture, One Shields Ave., University of California, Davis, CA, 95616, USA; 3 Department of Environmental Science, University of Parma, Parco Area delle Scienze 33a, 43100 Parma, Italy

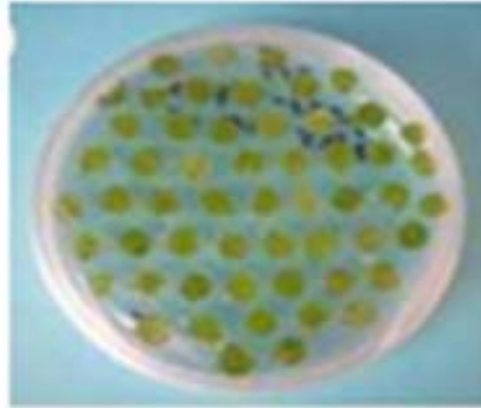
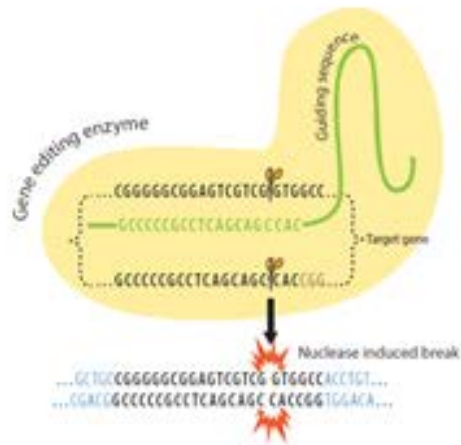
Field trials of RNAi-poplars



Sterility, normal growth of *LEAFY*-RNAi poplars



Overview of CRISPR methods in poplar

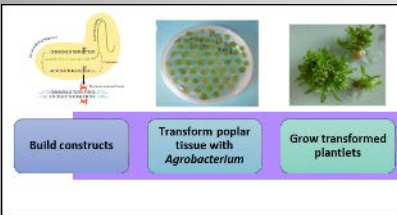


Build constructs

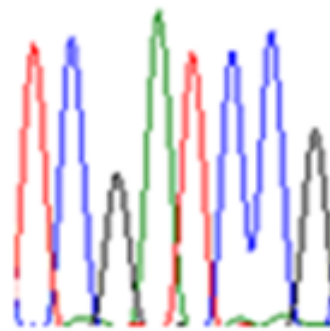
**Transform poplar
tissue with
*Agrobacterium***

**Grow transformed
plantlets**

Overview of CRISPR methods in poplar



TCGATCCG



DNA Sequences	Translated Protein Sequences
1. 7170_LFY-F2	CGT*****
2. 76-2_LFY-F2	CGT*****
3. 84-2_LFY-F2	CGT*****
4. 92_LFY-F2	CGT*****
5. 100_LFY-F2	CGT*****
6. 110_LFY-F2	CGT*****
7. 112-1_LFY-F2	CGT*****
8. 112-2_LFY-F2	CGT*****
9. 112-3_LFY-F2	CGT*****
10. 112-4_LFY-F2	CGT*****
11. 127-0_LFY-F2	CGT*****
12. 11-0F_LFY-F2	CGT*****
13. 12-1_LFY-F2	CGT*****
14. 12-2_LFY-F2	CGT*****
15. 13-3_LFY-F2	CGT*****
16. 120_LFY-F2	CGT*****
17. 240_202-F2	CGT*****
18. 126-1_LFY-F2	CGT*****
19. 126-3_LFY-F2	CGT*****
20. 127-1_LFY-F2	CGT*****
21. 127-3_LFY-F2	CGT*****

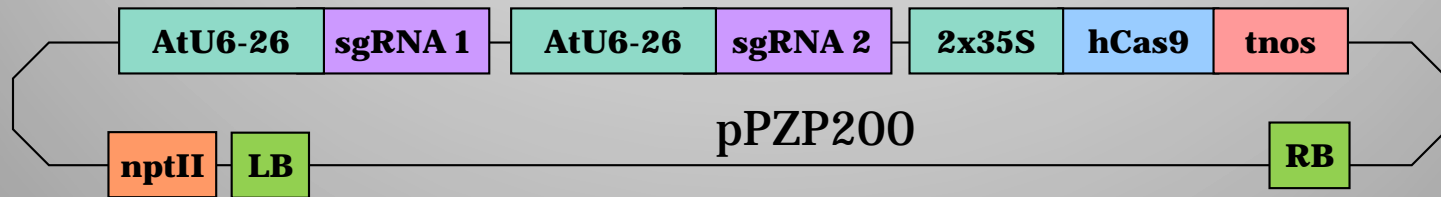
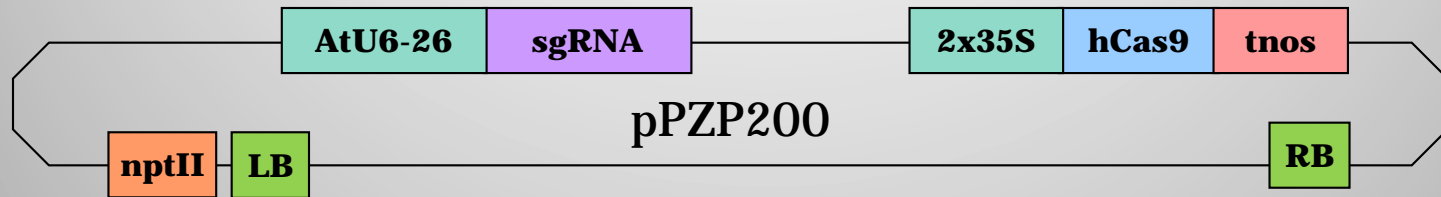
Extract DNA and gel-purify gene amplicons

Sequence amplicons across target sites

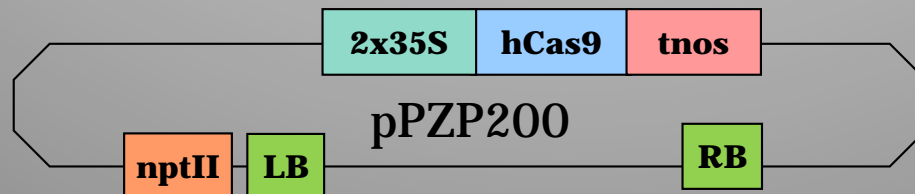
Identify mutation types and determine frequency

Experimental constructs – single and double targets per gene

Nuclease constructs



Control construct

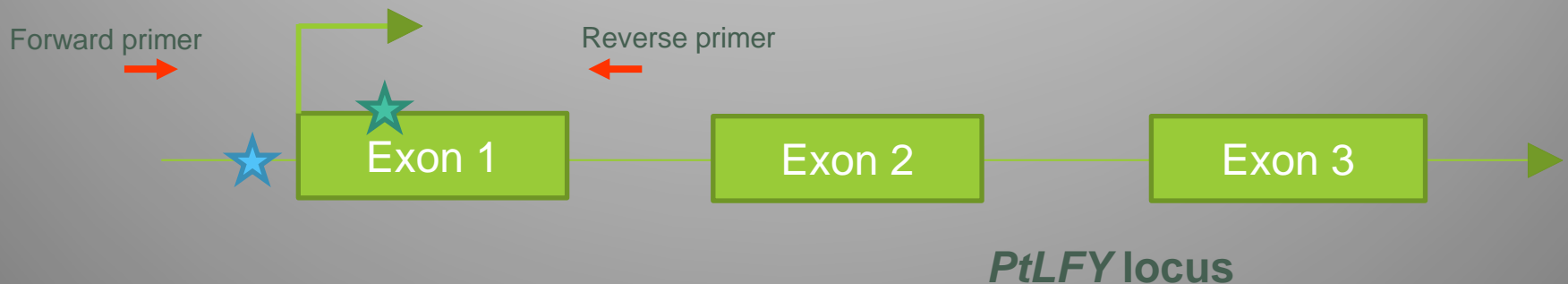


Targeting two sites in the single-copy LFY gene in poplar

>PtLFY
...CATGCACCAGTGAAAGATCACAGAGAGAGACAAGGGGGCAGATAGATATGGATCCGGAGGCTTT
CACGGCGAGTTTGTTCAAATGGGACACGAGAGCAATGGTGCCACATCCTAACCGTCTGCTTGAAATGGT
GCCCCGCCTCAGCAGCCACCGGCTGCGGCGTTTGCTGTAAGGCCAAGGGAGCTATGTGGGCTAGAGG
AGTTGTTTCAAGCTTATGGTATTAGGTACTACACGGCAGCGAAAATAGCTGAACTCGGGTTCACAGTGA...

Target site for LFYsg2

Target site for LFYsg1 (in exon 1)



Targeting two identical sites in the two paralogous AG genes in poplar

>PtAG1

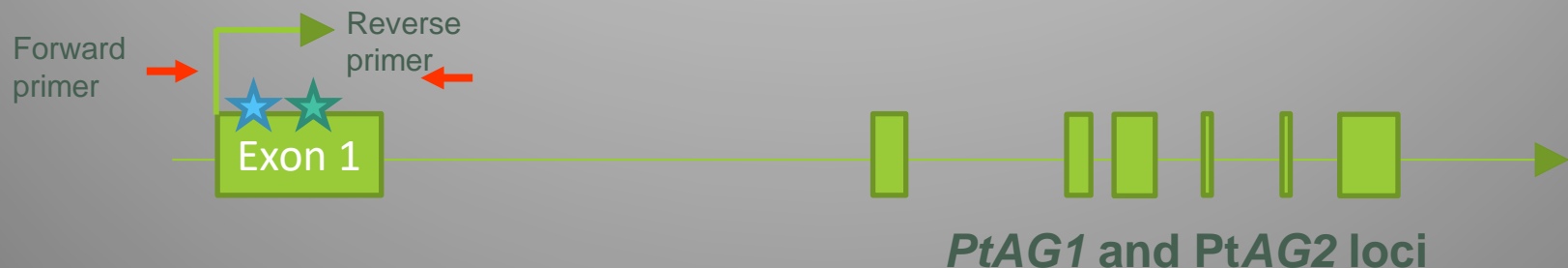
...GGATCAGCTAGCTAGACTGCAGCT**ATGG**AATATCAAATGAATCCCTTGAGAGCTCCCCCTGAGGAAGC
TAGGAAGGGGAAAGGTGGAGATCAAGCGGATCGAGAACACCACCAATCGCCAAGTCACTTTCTGCAA
AGGCGCAGTGGTTTGCTCAAGAAAGCCTACGAATTATCTGTTCTTTGCGATGCTGAGGTTGCACTCATCG...

Target site for AGsg2

Target site for AGsg1

>PtAG2

...GATCAGCTAGCTAGGCAGCAGCT**ATGG**CATACCAAATGAATCCCAAGAGAGCTCCCCCTGAGGAAGC
TGGGRAGGGGAAAGGTGGAGATCAAGCGGATCGAGAACACCACAAATCGYCAAGTCACTTTCTGCAA
AGGCGGAATGGTTTGCTCAAGAAAGCCTATGAATTATCTGTTCTTTGCGATGCTGAGGTTGCACTCATCG...



High mutation rates observed

- Cas9-only control events
 - No mutations
- CRISPR-Cas events
 - 80% with mutations
 - 50% knock-outs!

Large and small mutations – many routes to non-functioning gene

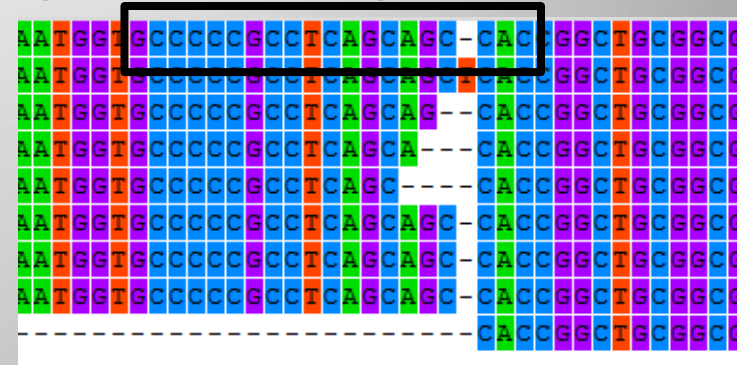
Mutations

Target site for LFYsg2

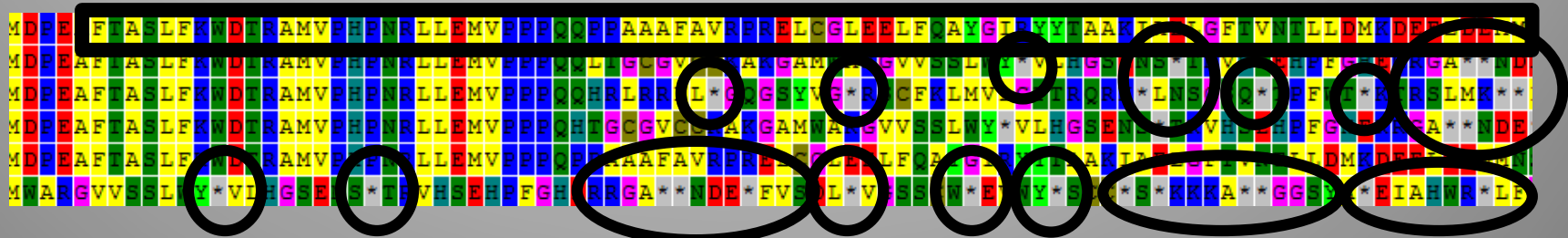
Target site for LFYsg1



...100bps...



Partial peptide sequence



Early stop codons

Agenda

1. Genetic engineering defined
2. Gene editing defined
3. Examples and applications
4. Our work with CRISPR
5. Society, regulation, markets

Social dimensions

- Largest barriers to use are social, not technical
- Ethics: Is the method OK to use at all?
 - Impossible to trace with certainty
 - Do we need to add tracer DNA?
- Should it be regulated and labeled like GMOs?
- Should all mutagenesis uses of gene editing be excused from regulation?
- What about market forces and self-labeling?

It's "too easy," need to restrict use by public?



2K



113



262

'Any idiot can do it.' Genome editor CRISPR could put mutant mice in everyone's reach

By [Jon Cohen](#) | Nov. 3, 2016 , 10:00 AM

In the beginning of 2013, Michael Wiles sat down with high-level managers of the Jackson Laboratory in Bar Harbor, Maine, and told them about a novel way to cut DNA that had amazing power. The lab, called JAX for short, genetically engineers mice that it sells to researchers under a trademarked brand: JAX® Mice, it likes to boast, "are the highest quality and most-published mouse models in the world." Wiles evaluates and develops technologies for the lab, and he was convinced that this new strategy that bacteria and archaea use to protect the way JAX engineered mice. "Of about a dozen



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26



162



A Swedish scientist included cabbage engineered with CRISPR-Cas9 in this pasta dish.

Stefan Jansson/Umeå University

Did a Swedish researcher eat the first CRISPR meal ever served?

By [Jon Cohen](#) | Sep. 7, 2016, 2:30 PM

In what Swedish plant scientist Stefan Jansson declares “maybe” a historic event, he cultivated, grew, and ate a plant that had its genome edited with CRISPR-Cas9. Umeå University, where Jansson studies how trees know it’s autumn and how proteins allow plants to harvest light, released a 5 September [press release](#) about his meal, a pasta dish that included 300 grams of cabbage he grew from seeds that had been genetically modified with CRISPR-

Major wars over CRISPR patents ongoing

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University of California attorney Todd Walters addresses the three judges presiding over the CRISPR patent hearing. Dana Verkouteren

CRISPR patent hearing produces no clear winner, only 'soft signals'

By Jon Cohen | Dec. 6, 2016, 6:00 PM

Global regulatory quandry

PUBLISHED: 8 JANUARY 2015 | ARTICLE NUMBER: 14011 | DOI: 10.1038/NPLANTS.2014.11

comment

Regulatory uncertainty over genome editing

Huw D. Jones

Genome editing opens up opportunities for the precise and rapid alteration of crops to boost yields, protect against pests and diseases and enhance nutrient content. The extent to which applied plant research and crop breeding benefit will depend on how the EU decides to regulate this fledgling technology.

We are at the dawn of a new paradigm in plant breeding. Classical approaches to crop improvement based on hybridization and selection can now be complemented by targeted genome editing that exploits knowledge of specific gene sequences in a systematic way. Unlike conventional genetic modification that results from the insertion of large pieces of exogenous DNA,

or maize renders the plants highly resistant to lepidopteron pests; these lepidopteron-resistant crops are grown around the world. However, this technique cannot be used to make small edits to existing genes, and can lead to the random disruption of native genes because the destination of the inserted DNA cannot be dictated.

In contrast to traditional genetic modification, genome editing makes use of

one or a few bases at the cut site, resulting in a mutation. Mutations generated in this way are indistinguishable from those that occur naturally and drive evolution, as well as from those induced through the application of chemical mutagens or radiation, as employed in mutation breeding programmes since the 1940s.

Here, I focus on the potential applications and regulation of this simple 'cut and repair'

USA GMO labeling law in place

[Home](#) > [News Center](#) > [New US Labeling Law for Bioengineered Foods](#)

NEW US LABELING LAW FOR BIOENGINEERED FOODS

September 28, 2016



Building on the work of individual states in preparing and implementing laws to regulate the labeling of bioengineered foods, the USA has enacted a federal law providing countrywide protection and consistency for consumers.

Jim Cook, SGS Food Scientific and Regulatory Affairs Manager explains in more detail.

On July 1, 2016, the USA's first labeling law, the Vermont Genetically Engineered (GE) food labeling law Act 120 became effective but as of July 29 when President Obama signed the National Bioengineering Food Disclosure Law ¹ it

New USDA proposal on Jan 19, 2017



7008

Federal Register / Vol. 82, No. 12 / Thursday, January 19, 2017 / Proposed Rules

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

7 CFR Part 340

[Docket No. APHIS–2015–0057]

RIN 0579–AE15

Importation, Interstate Movement, and Environmental Release of Certain Genetically Engineered Organisms

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Proposed rule.

SUMMARY: APHIS is proposing to revise its regulations regarding the importation, interstate movement, and environmental release of certain genetically engineered organisms in order to update the regulations in response to advances in genetic engineering and understanding of the plant pest and noxious weed risk posed by genetically engineered (GE) organisms, thereby reducing burden for regulated entities whose organisms pose no plant pest or noxious weed risks. This would be the first comprehensive revision of the regulations since they were established in 1987.

DATES: We will consider all comments that we receive on or before May 19, 2017.

SUPPLEMENTARY INFORMATION:

Background

Overview of the Current Regulations

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) administers regulations in 7 CFR part 340, “Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests” (referred to below as the regulations). The current regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered (GE) organisms that are considered “regulated articles.”

Under the current regulations, a GE organism is considered to be a regulated article if the donor organism, recipient organism, vector, or vector agent¹ is a plant pest or if the Administrator has reason to believe the GE organism is a plant pest. A *plant pest* is defined in § 340.1 as “Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or

article pursuant to 7 CFR part 340.

Agency Actions Following Promulgation of the Current Regulations

APHIS first issued these regulations in 1987 under the authority of the Federal Plant Pest Act of 1957 (FPPA) and the Plant Quarantine Act of 1912 (PQA), two acts that were subsumed into the Plant Protection Act (PPA, 7 U.S.C. 7701 *et seq.*) in 2000, along with other provisions. Since 1987, APHIS has amended the regulations six times, in 1988, 1990, 1993, 1994, 1997, and 2005, to institute exemptions from permitting for certain microorganisms and *Arabidopsis*, to institute the notification, petition, and extension procedures referenced above, and to exclude plants engineered to produce industrial compounds from the notification process.

Although, as discussed above, the current regulations have various functions, their primary function to date has been as a means for APHIS to authorize the importation, interstate movement, and introduction of certain GE organisms via the permit and notification procedures referred to above. Permits and notifications are collectively known as “authorizations.” To date, APHIS has issued more than 18,000 authorizations for the environmental release of GE organisms in multiple sites, primarily for research and development of improved crop varieties for agriculture. Additionally, APHIS has issued more than 12,000

Gene editing is GE...

- “For the purposes of this rule, APHIS is proposing to consider genome editing to be within the definition of genetic engineering. “
- Does that mean every editing line must come before USDA?
- How much data and confinement is required during research and breeding?
- Is it essentially a GMO?

But....the definition of GE is further restricted...

- ... an organism will not be considered a genetically engineered organism if:
- (1) The genetic modification to the organism is solely a deletion of any size or a single base pair substitution which could otherwise be obtained through the use of chemical- or radiation-based mutagenesis; or

But....the definition of GE is further restricted...

- ... an organism will not be considered a genetically engineered organism if:
- (2) The genetic modification to the organism is solely introducing only naturally occurring nucleic acid sequences from a sexually compatible relative that could otherwise cross with the recipient organism and produce viable progeny through traditional breeding...or

But....the definition of GE is complex

- ... an organism will not be considered a genetically engineered organism if:
- (3) The organism is a “null segregant,” that is, the progeny of a GE organism where the only genetic modification was the insertion of donor nucleic acid into the recipient’s genome, but the donor nucleic acid is not passed to the recipient organism’s progeny

What does this mean?

- “For the purposes of this rule, APHIS is proposing to consider genome editing to be within the definition of genetic engineering. “
- Does that mean every editing line must come before USDA?
- How much data and confinement is required during research and breeding?
- Is it essentially a GMO or not?

ASTA happy so far



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ASTA's overarching policy is that plant varieties developed through the latest breeding methods should not be differentially regulated if they are similar to or indistinguishable from varieties that could have been produced through earlier breeding methods.

ASTA WELCOMES DIRECTION OF PLANT BREEDING POLICY FROM USDA

 Posted by bshively@amseed.org  On January 18, 2017  0 Comments

Alexandria, Va.—January 18, 2017—USDA today released for pre-publication a proposed rule-making notice related to plant breeding innovation. The American Seed Trade Association (ASTA) has been actively engaged in discussions with the agency throughout the rule-making process, and is pleased that the proposal recognizes plant breeders' long track record of safety and quality.

"The farm and food value chain is committed to innovating in a responsible and sustainable way," said ASTA President & CEO Andy LaVigne. "We look forward to continuing these conversations with the Trump Administration to ensure sound policy that fosters continued innovation and promotes the movement of seed and other agricultural products around world."

ASTA's overarching policy is that plant varieties developed through the latest breeding methods should not be differentially regulated if they are similar to or indistinguishable from varieties that could have been produced through earlier breeding methods.

Regulation of gene editing depends on decisions of three agencies – coordinated but distinct



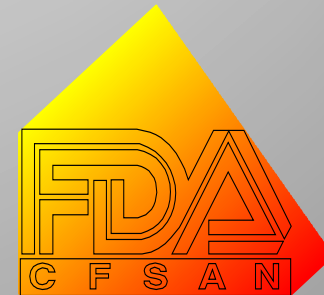
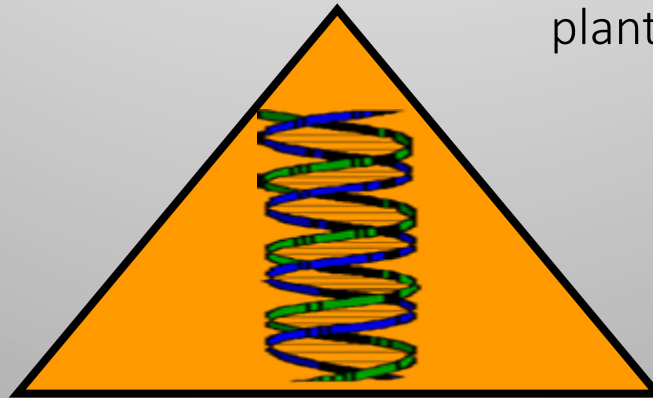
USDA – US Department of Agriculture:

Agricultural products of plant or animal origin



EPA - Environmental Protection Agency:

Biopesticides



FDA - Food & Drug Administration:

Food consumed by humans or animals

FDA is
proposing to
regulate gene
edited animals
as animal
“drugs”

#187

Guidance for Industry
Regulation of Intentionally Altered
Genomic DNA in Animals

Draft Guidance

(This guidance is a revision of Guidance #187, “Regulation of Genetically Engineered Animals,” which has been revised to update information concerning the products of different technologies used to produce such animals, and to provide new weblinks.)

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to <http://www.regulations.gov>. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with the Docket No. FDA-2008-D-0394.

For further information regarding this document, contact [Laura R. Epstein](mailto:Laura.R.Epstein@fda.hhs.gov), Center for Veterinary Medicine (HFV-1), Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, 301-796-8558, email: Laura.Epstein@fda.hhs.gov.

Additional copies of this guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either <http://www.fda.gov/AnimalVeterinary/default.htm> or <http://www.regulations.gov>.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
January 2017

Markets are another thing....

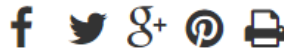
The National Organic Standard Boards has **banned** gene editing technologies

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Organic board bans gene editing technology

CATTLE AND BEEF INDUSTRY NEWS

NOV 25, 2016 By **KERRY HALLADAY, WLJ MANAGING EDITOR**



When a government agency describes something as causing the “demise” of species and displacing Americans, they must surely be describing a foreign enemy, right? Or maybe some pandemic plaguing the countryside?

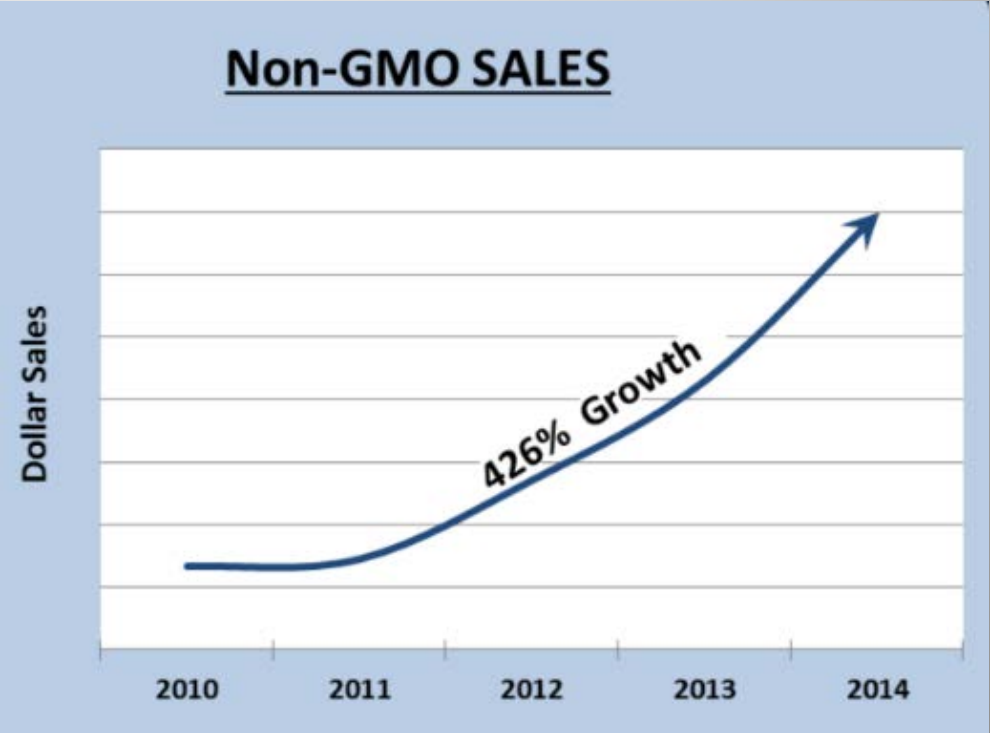
Apparently not. To the potential dismay of the National Organic Standards Board (NOSB) users, the board would, among other things, ban plants and animals from being considered organic. Also, genetic engineering—an “excluded method” of organic production—additionally attributed many alarm

“Every organic stakeholder is clear on the need to protect organic integrity. Every effort must be made to ensure that organic products remain free of genetic engineering.”

Among other things, the proposal would ban the use of Cas 9, Zinc Finger Nuclease (ZFN), and other genetic engineering for the purposes of organic production. The NOSB also listed “excluded methods” of organic production.

“Every organic stakeholder is clear that genetic engineering is an imminent threat to organic integrity. Every effort must be made to protect that integrity,”

Proliferation of self-defined no-GMO labels likely to exclude gene editing?



<https://www.aphis.usda.gov/stakeholders/downloads/2015/coexistence/Errol-Schweizer.pdf>

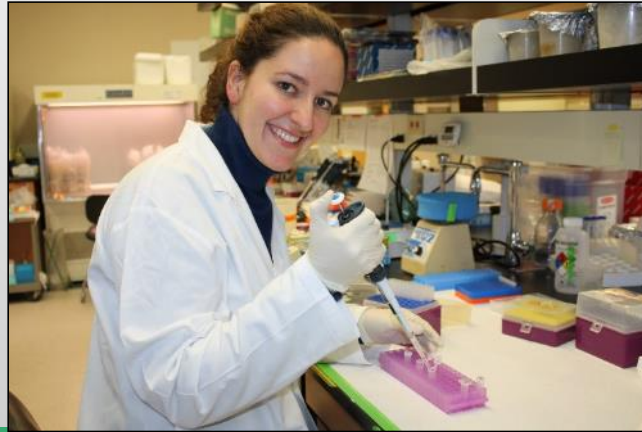
Summary

- Gene editing works well everywhere its been tried
 - This one is not hype!
- Still depends on capability for GE
 - Difficult in many species and genotypes
- Significant social issues and uncertainties
 - Ethics, regulation, market exclusions, labeling, and patents
 - Key determinants of whether this technology will matter a lot or not so much
- Could be a powerful tool for crops, including mint?
 - Key constraints are science knowledge, genetic engineering capability, and regulation/markets

Thanks



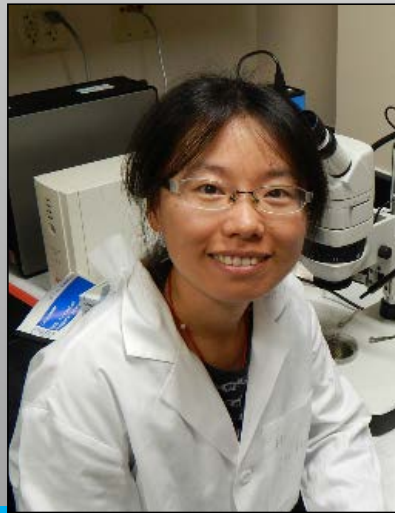
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Stef Elorriaga



Amy Klocko



Haiwei Lu



Flavia Tussulini



Clark Embleton,
High School intern

