Gene editing for crop improvement

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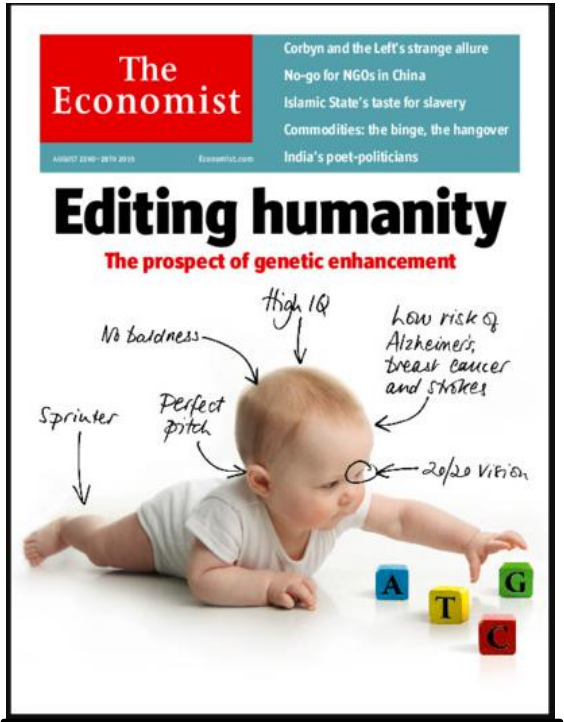
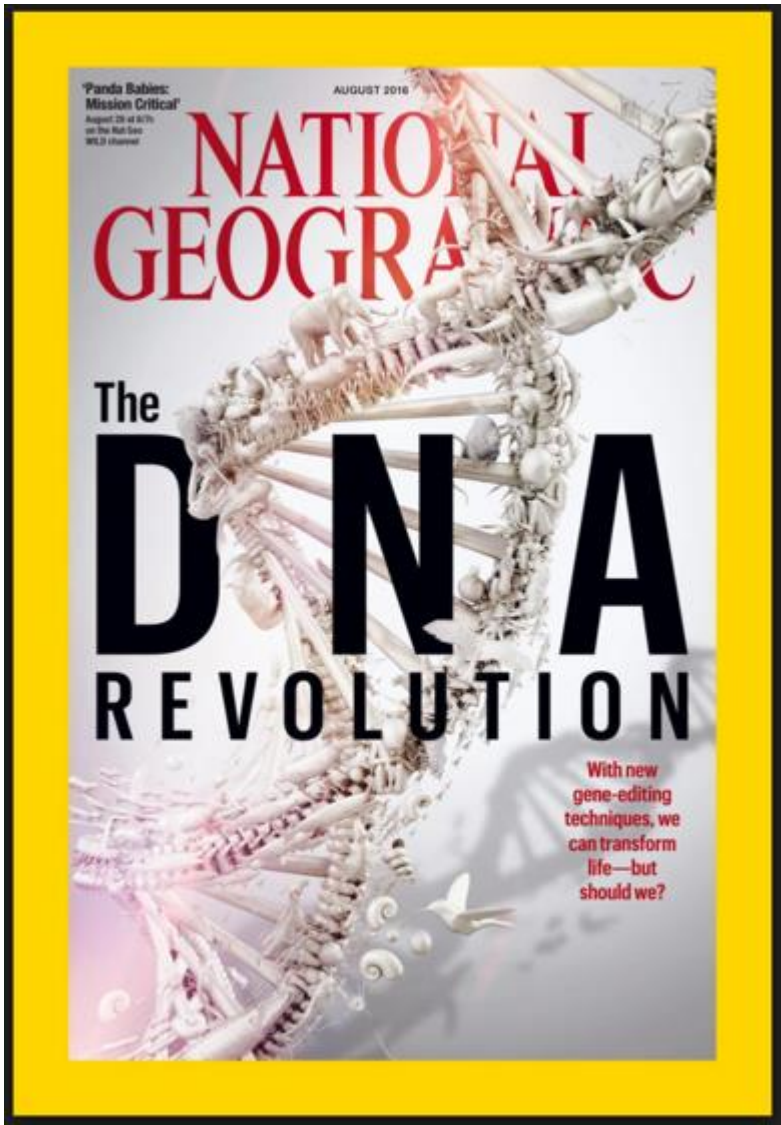
TEXAS A&M UNIVERSITY

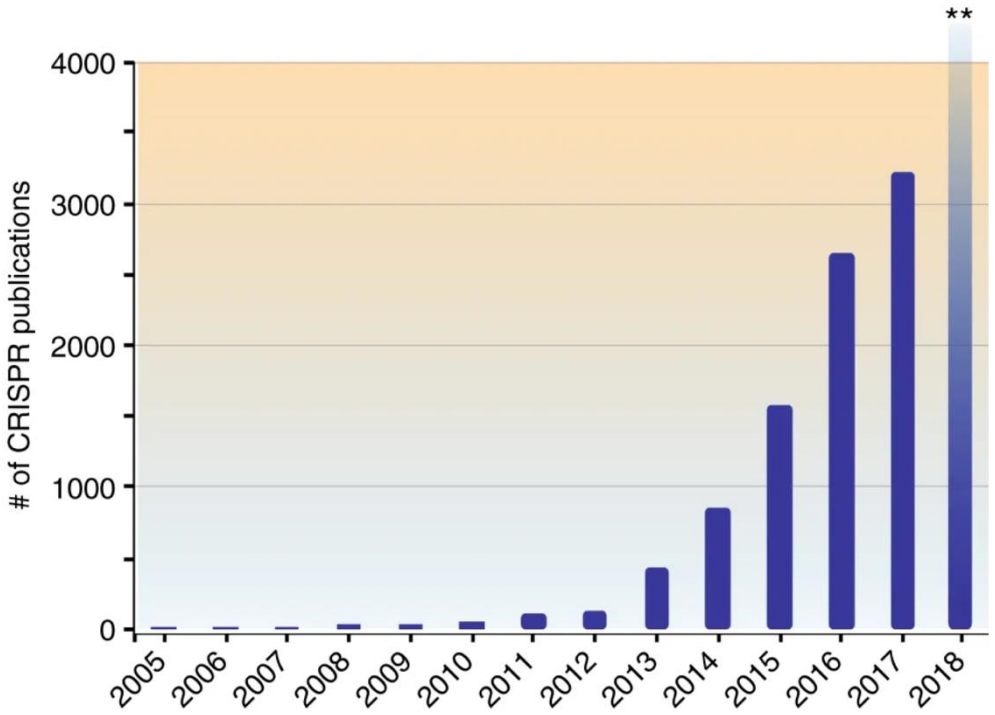
"CRISPR Everywhere"

The rise of gene editing

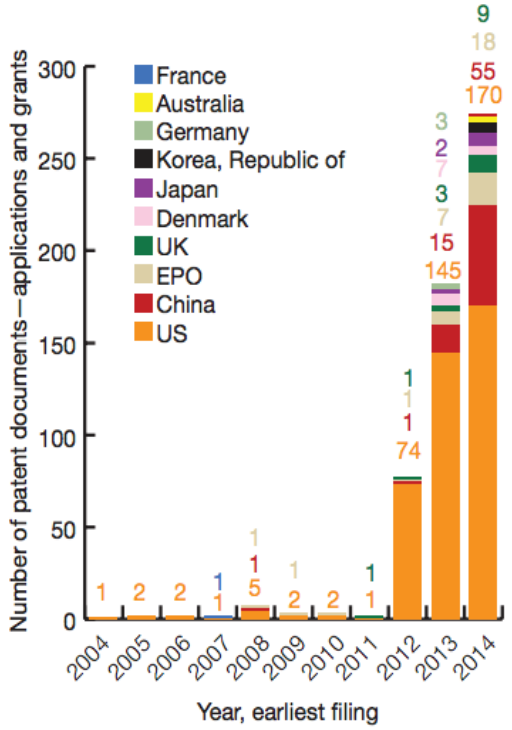




Explosion of CRISPR research began in 2012



Number of CRISPR publications per year



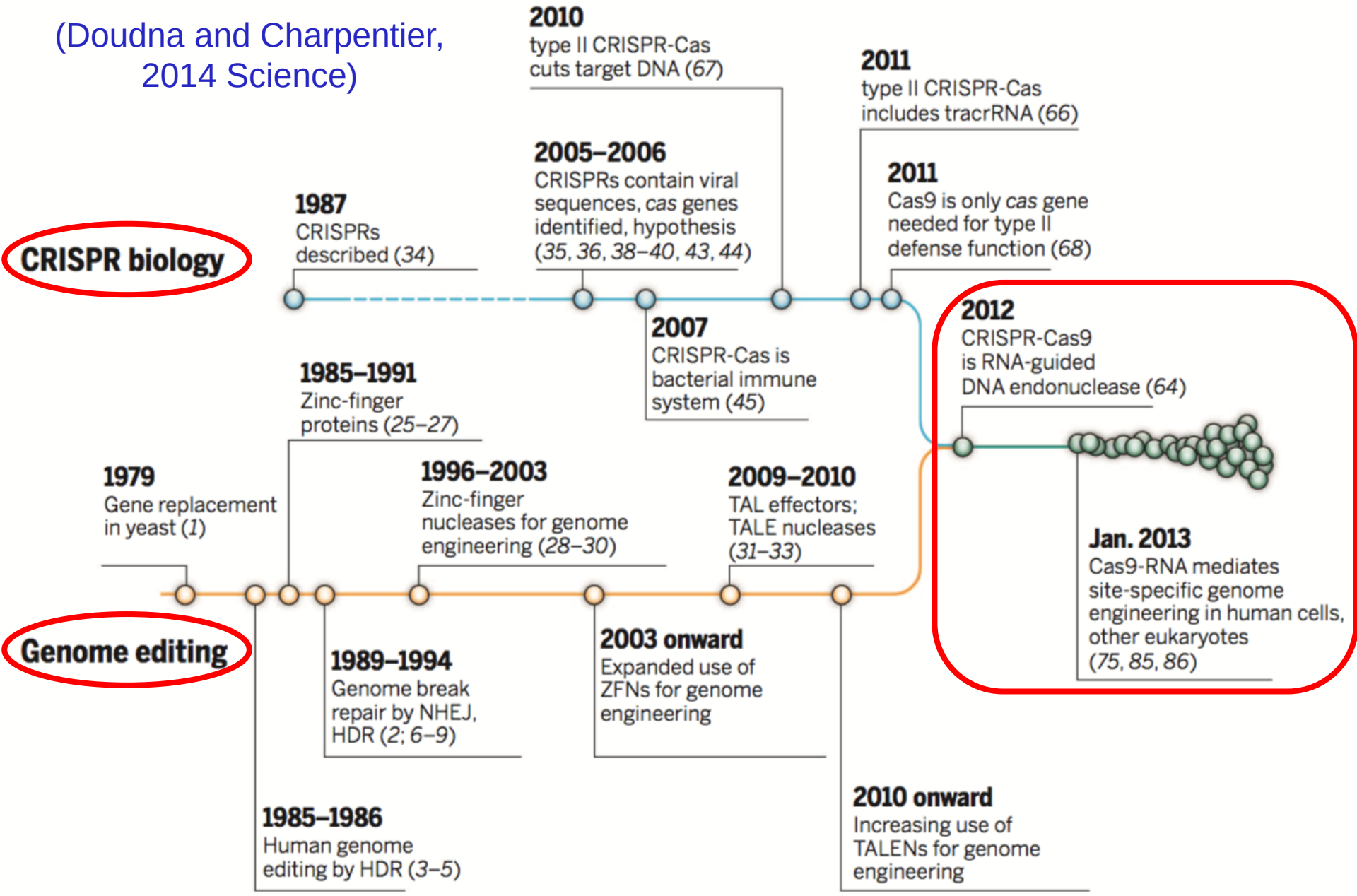
Number of CRISPR patents per year

CRISPR now impacting a wide range of fields:

- Basic biological research and functional genomics
- Medical research and human therapeutics
- Viral, bacterial, plant and animal research

CRISPR biology meets genome editing

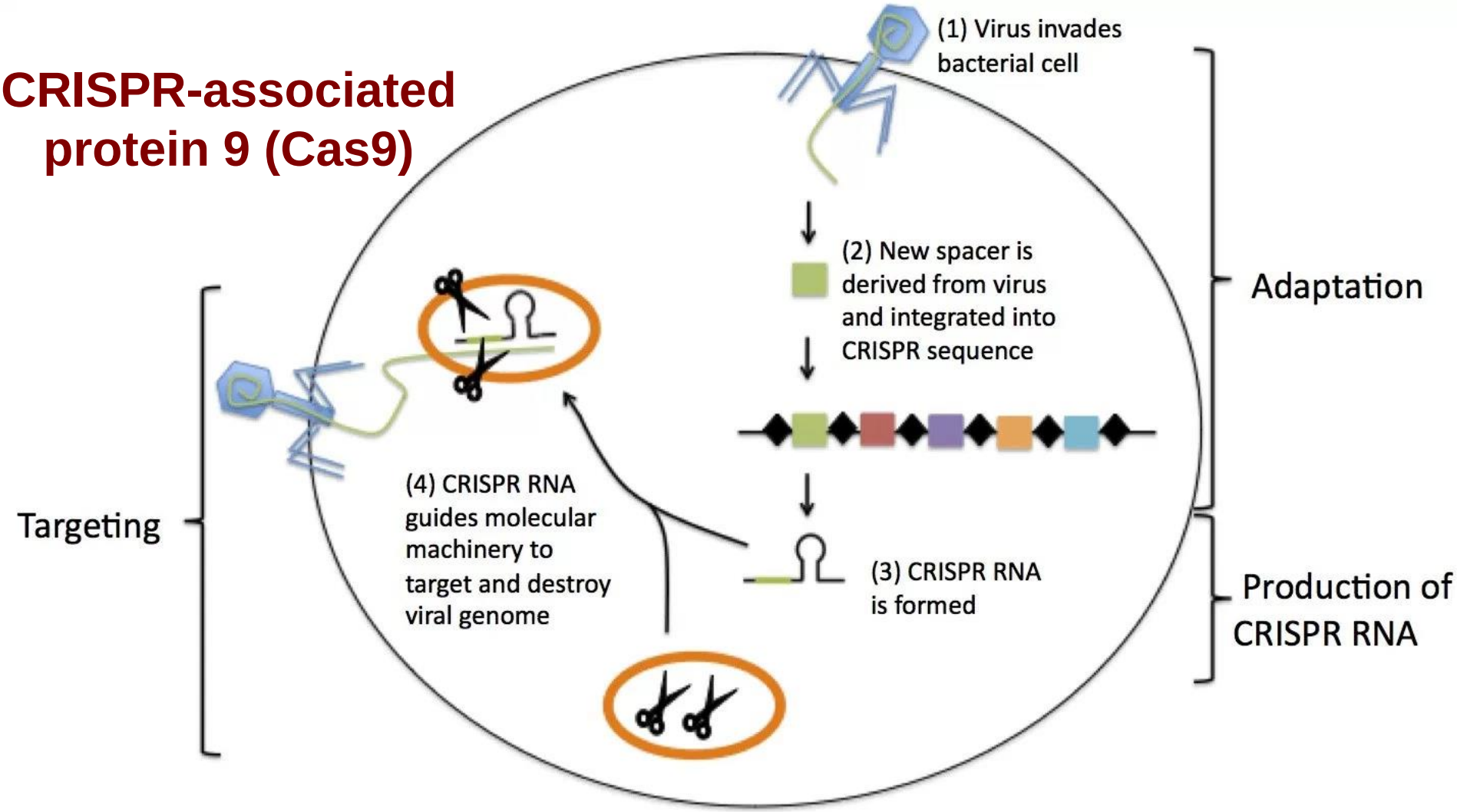
(Doudna and Charpentier, 2014 Science)



CRISPR bacterial immune system from viruses

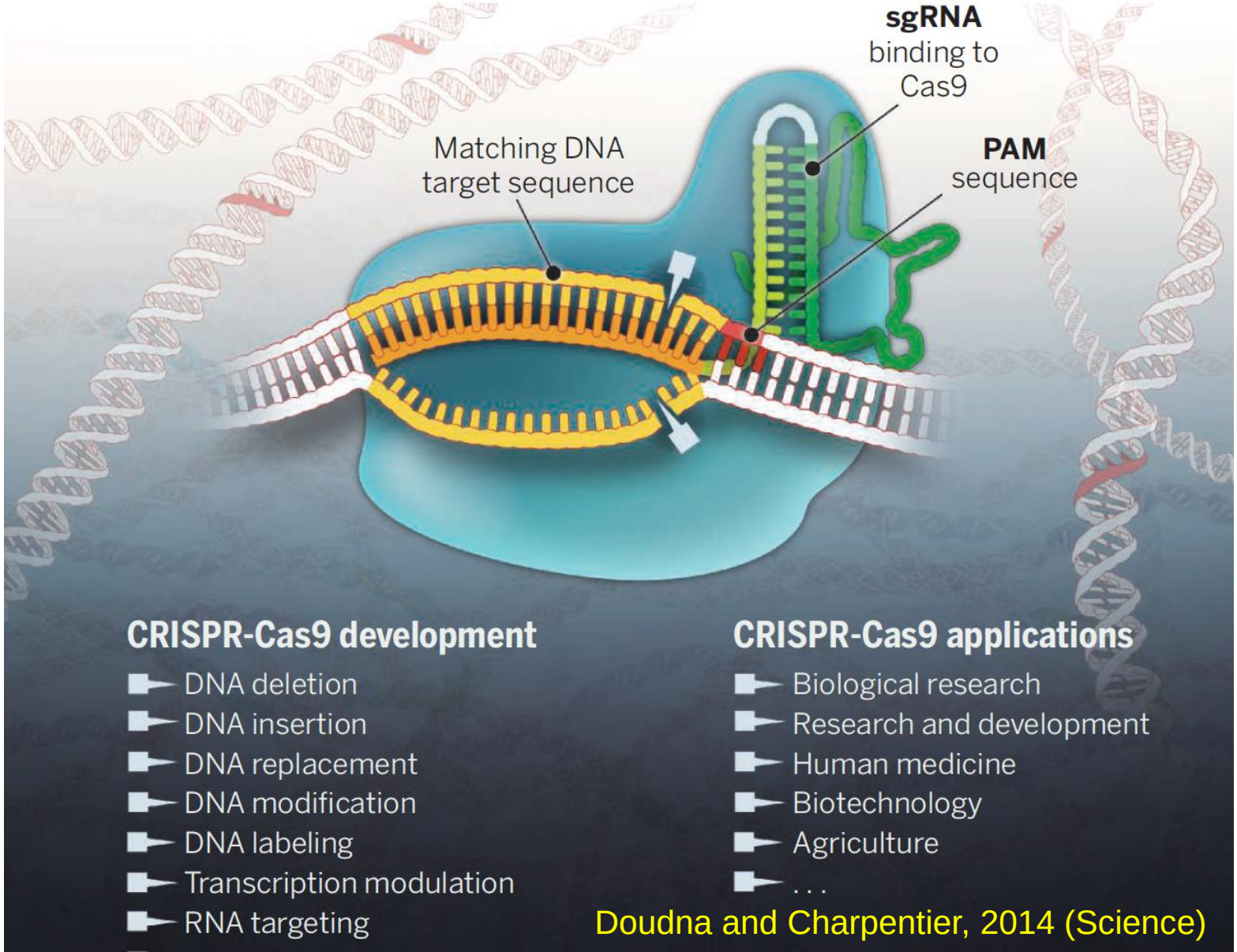
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

CRISPR-associated protein 9 (Cas9)



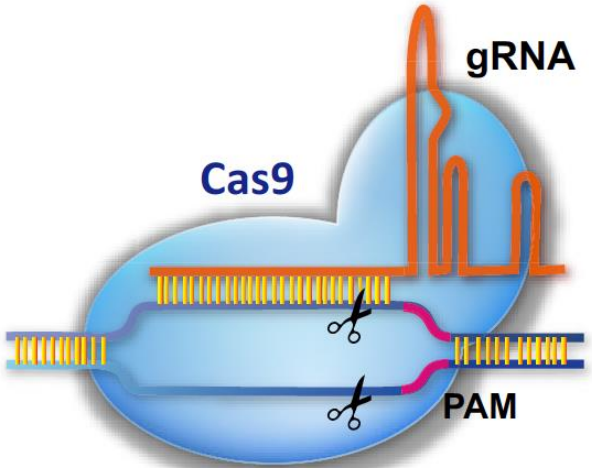
http://sitn.hms.harvard.edu/flash/2014/crispr-a-game-changing-genetic-engineering-technique/

New tool for crop improvement:
CRISPR-Cas9 genome editing



Doudna and Charpentier, 2014 (Science)

CRISPR/Cas9 genome editing in plants: types of mutations



Double strand break (DSB)

Non-homologous end joining

Homology-directed recombination

NHEJ

HR

no donor DNA

+ donor DNA

+ donor DNA

+ donor DNA

gene knockout

(a)

gene insertion

(b)

gene modification

(c)

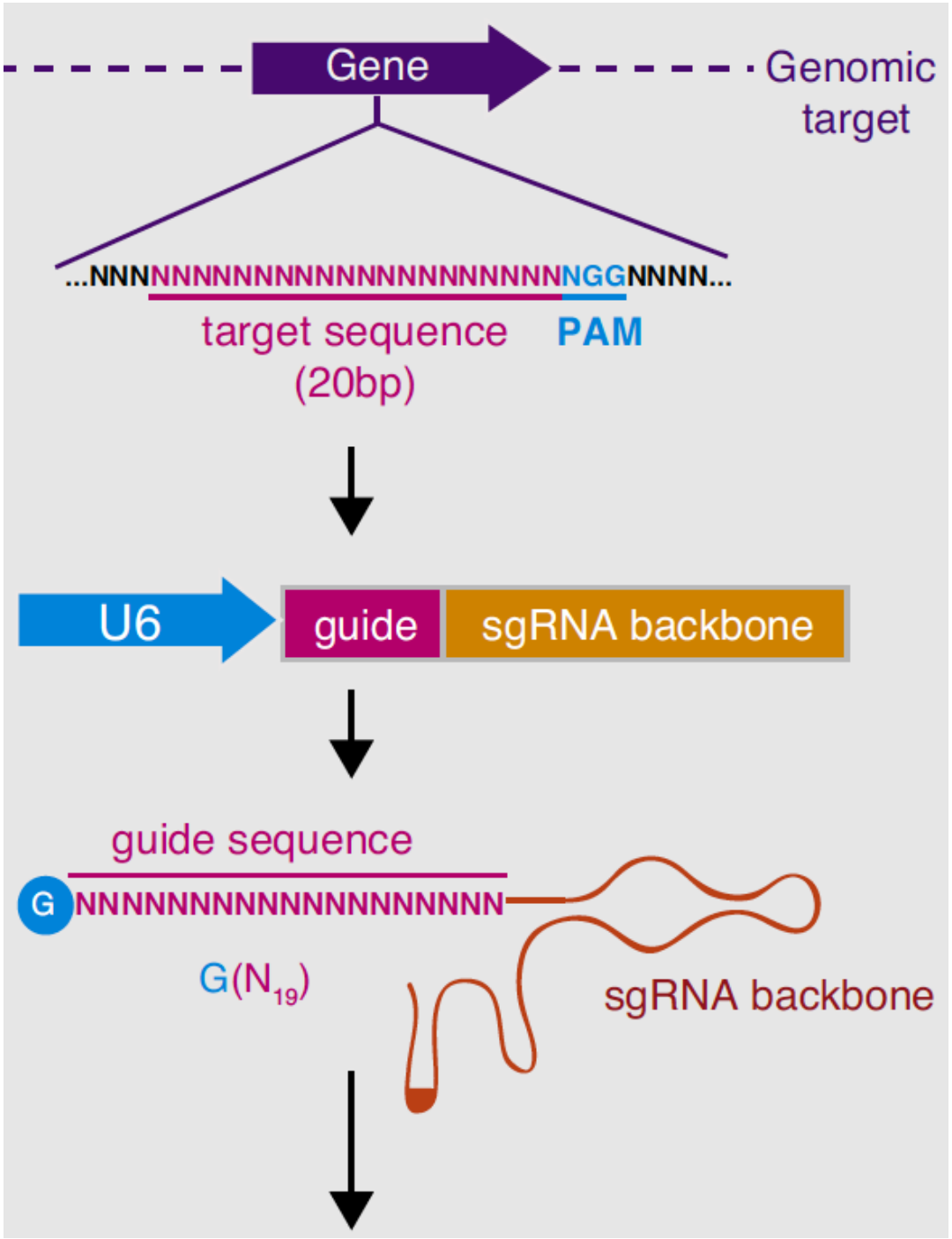
gene insertion

(d)

Bortesi and Fischer, 2015 Biotech. Advances

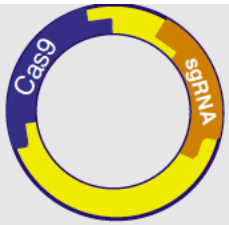
CRISPR-Cas9 genome editing process

1. **Select genomic target**
   - 20 bp sequence + PAM (NGG)
   - Online tools to avoid off-targets
2. **Design sgRNA**
   - Express sgRNA with small RNA promoter
   - Guide sequence matches target



Belhaj et al. (2015) Curr Opin Biotech

3. Assemble Cas9 / sgRNA construct



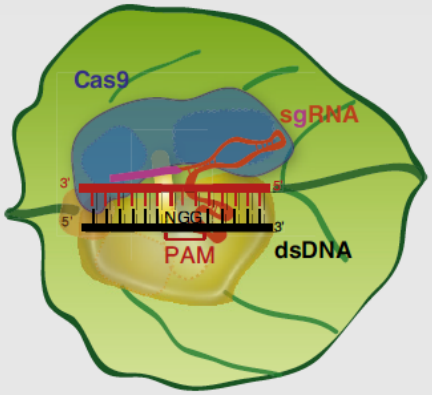
Assembly of sgRNA and Cas9

4. Deliver to plants

a. Protoplast tranformation

b. Agrobacterium transformation

c. Callus bombardment



Delivery to plants

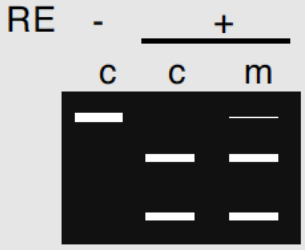
5. Regenerate and screen transgenic plants for gene editing events

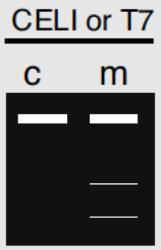
Screening for mutants

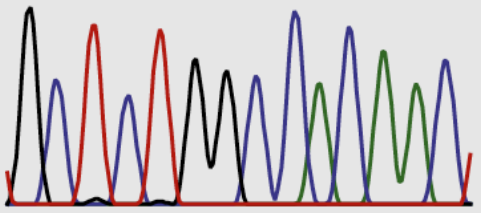
(a) Restriction Enzyme Site Loss

(b) Surveyor assay

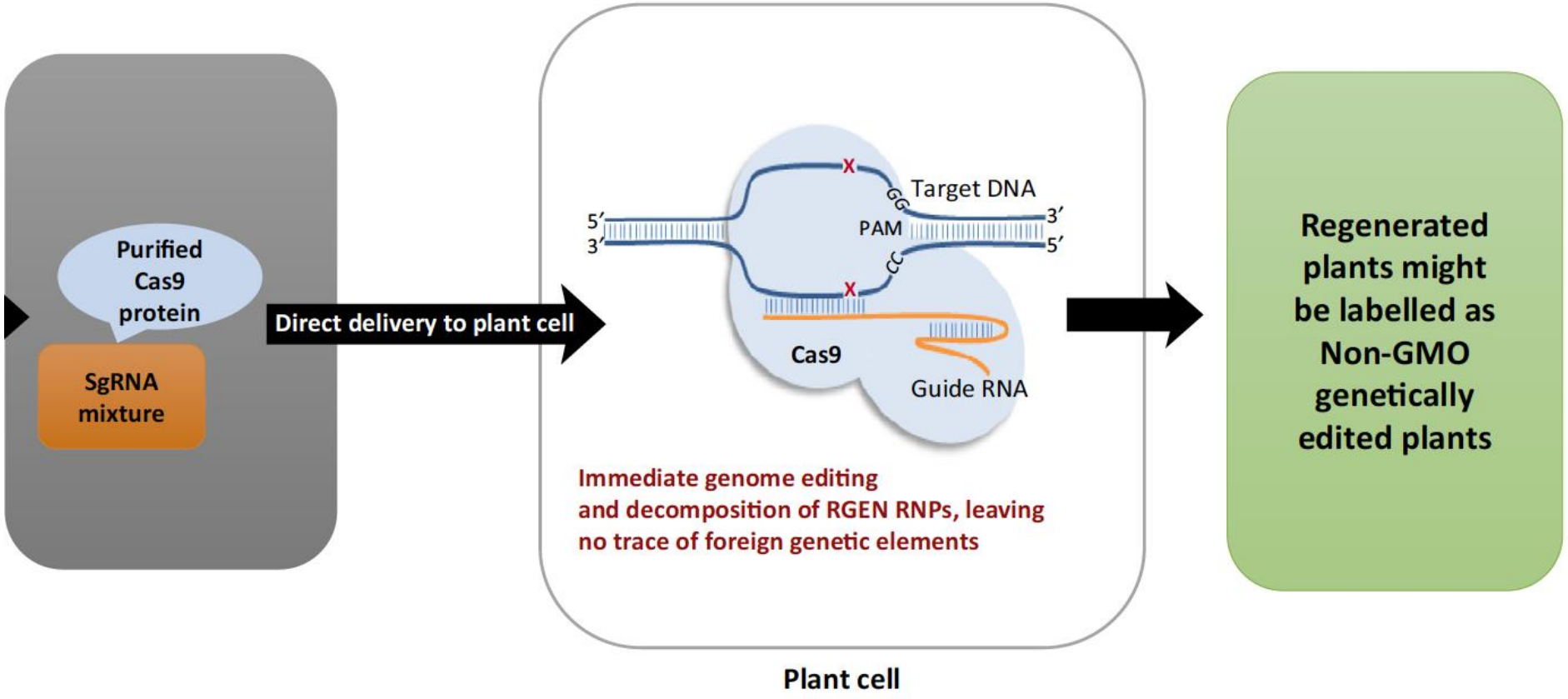
(c) Next Generation Sequencing (NGS)







Ribonucleoprotein (RNP) direct delivery of CRISPR/Cas9 for “DNA-free” genome editing

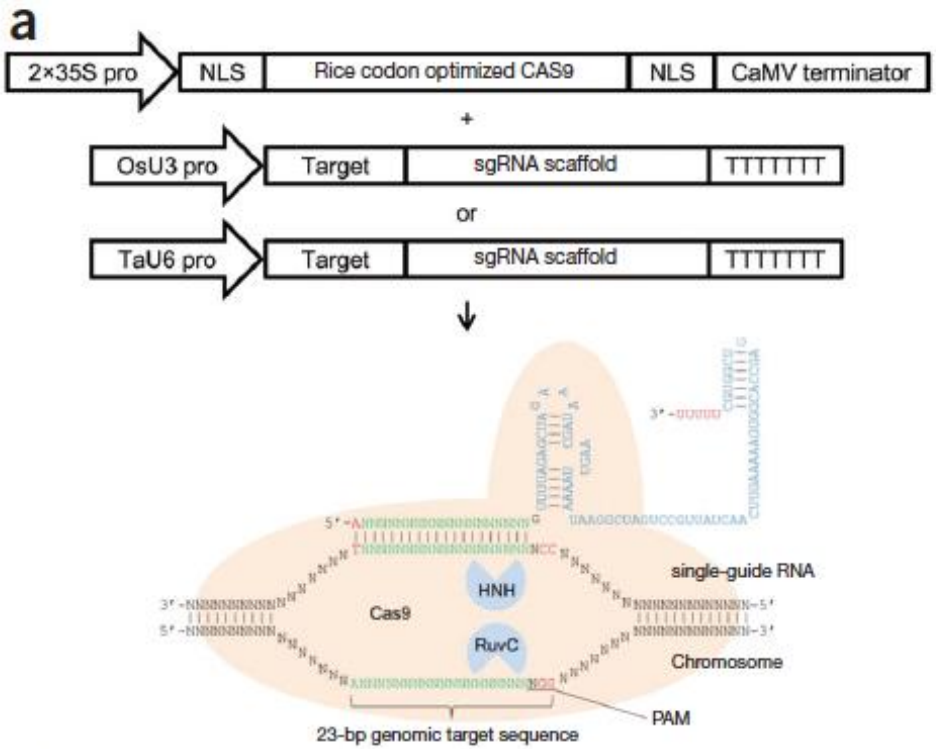


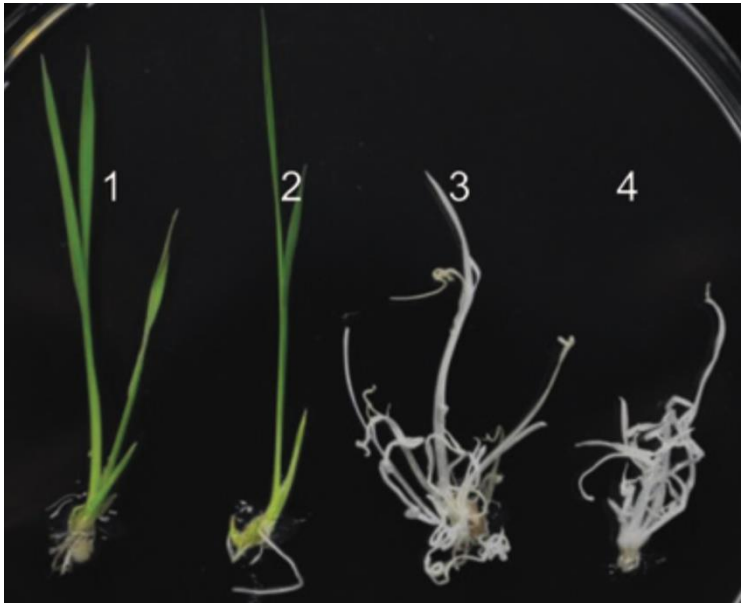
TRENDS in Biotechnology

Kanchiswamy et al. 2015

Example of gene knockout in rice: OsPDS gene

Knocking out the rice phytoene desaturase gene gives a dwarf and albino phenotype



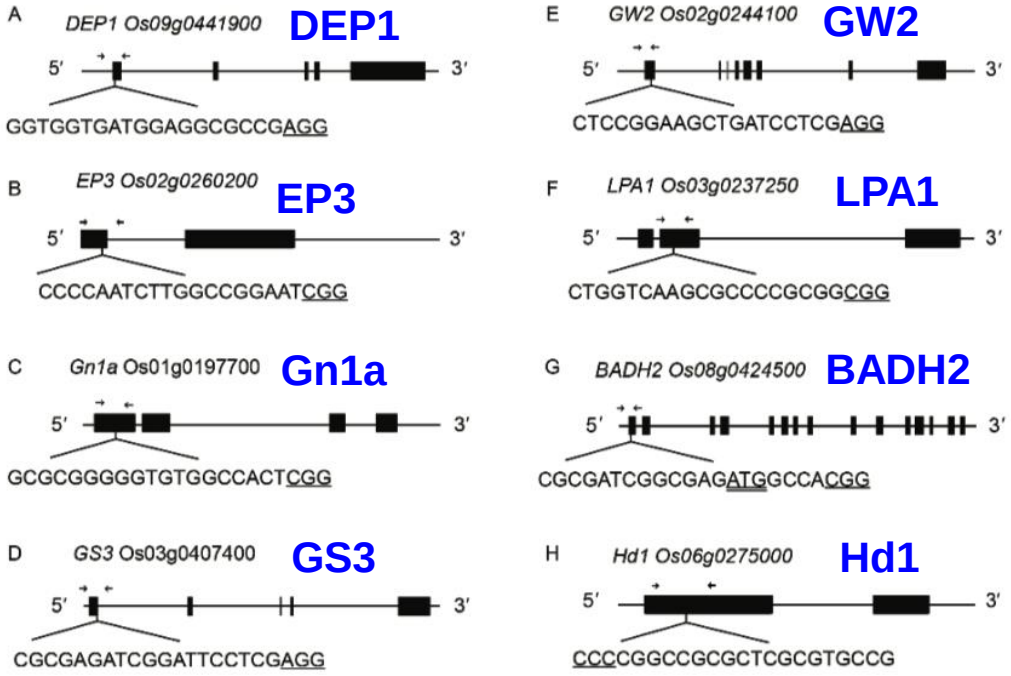


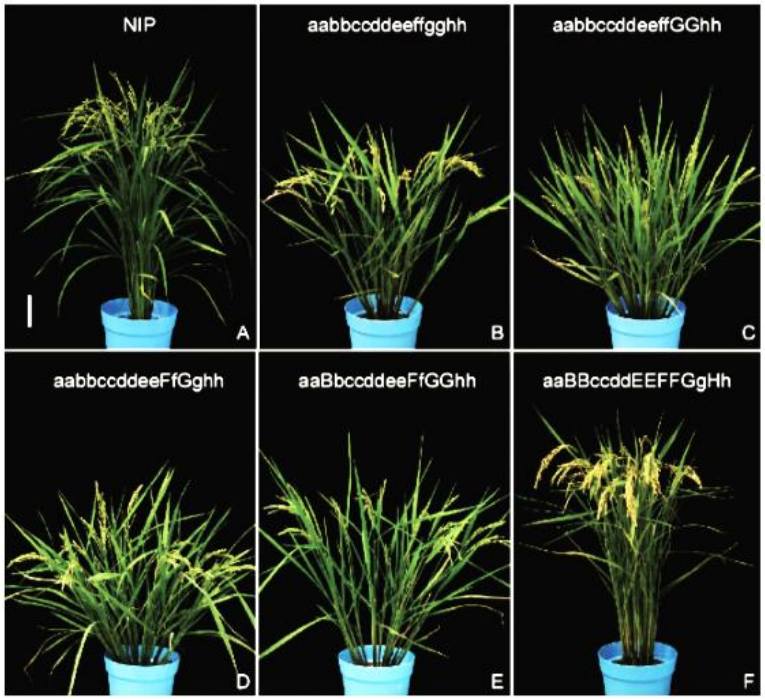
Shan et al (2013) Nat. Biotech.

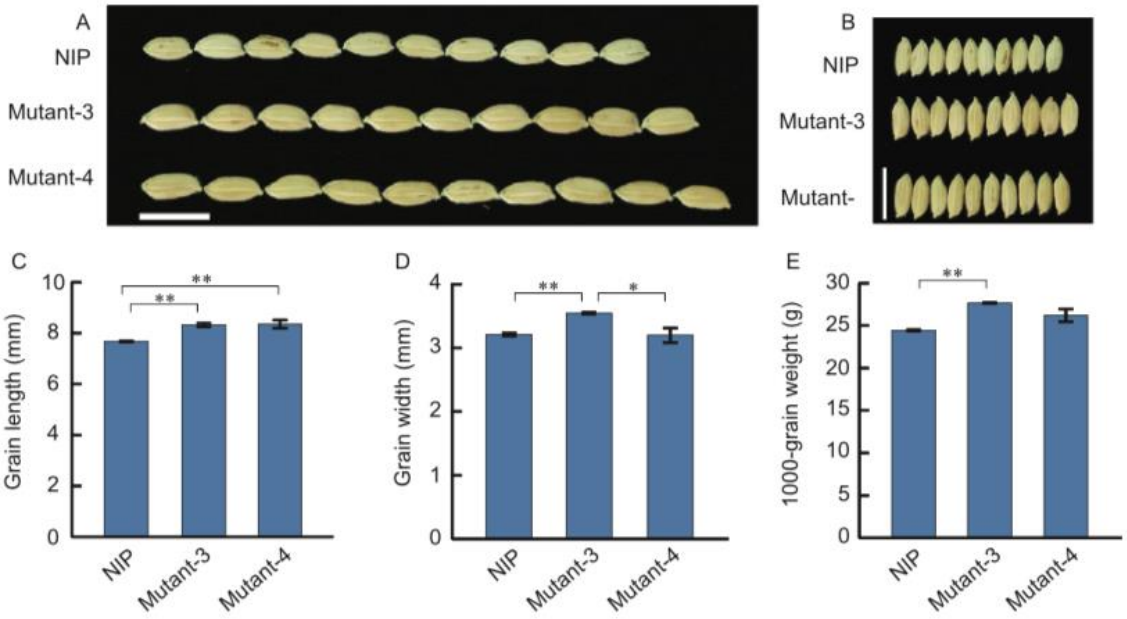
Multiplex gene editing in rice

Shen et al. 2017

DEP1, EP3, Gn1a, GS3, GW2, LPA1, BADH2, Hd1







Future gene-edited products using TALEN technology

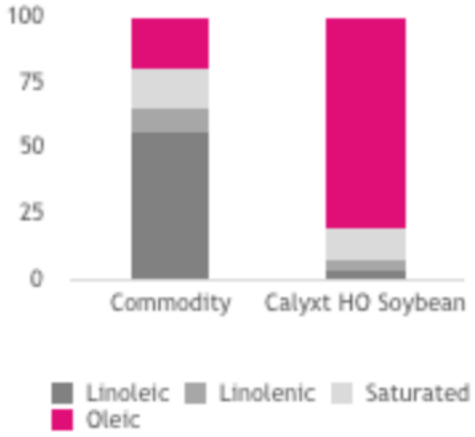
Plant Biotechnology Journal

Plant Biotechnology Journal (2014) **12**, pp. 934–940 doi: 10.1111/pbi.12201

Dan Voytas,
Chief Science Officer,
Calyxt

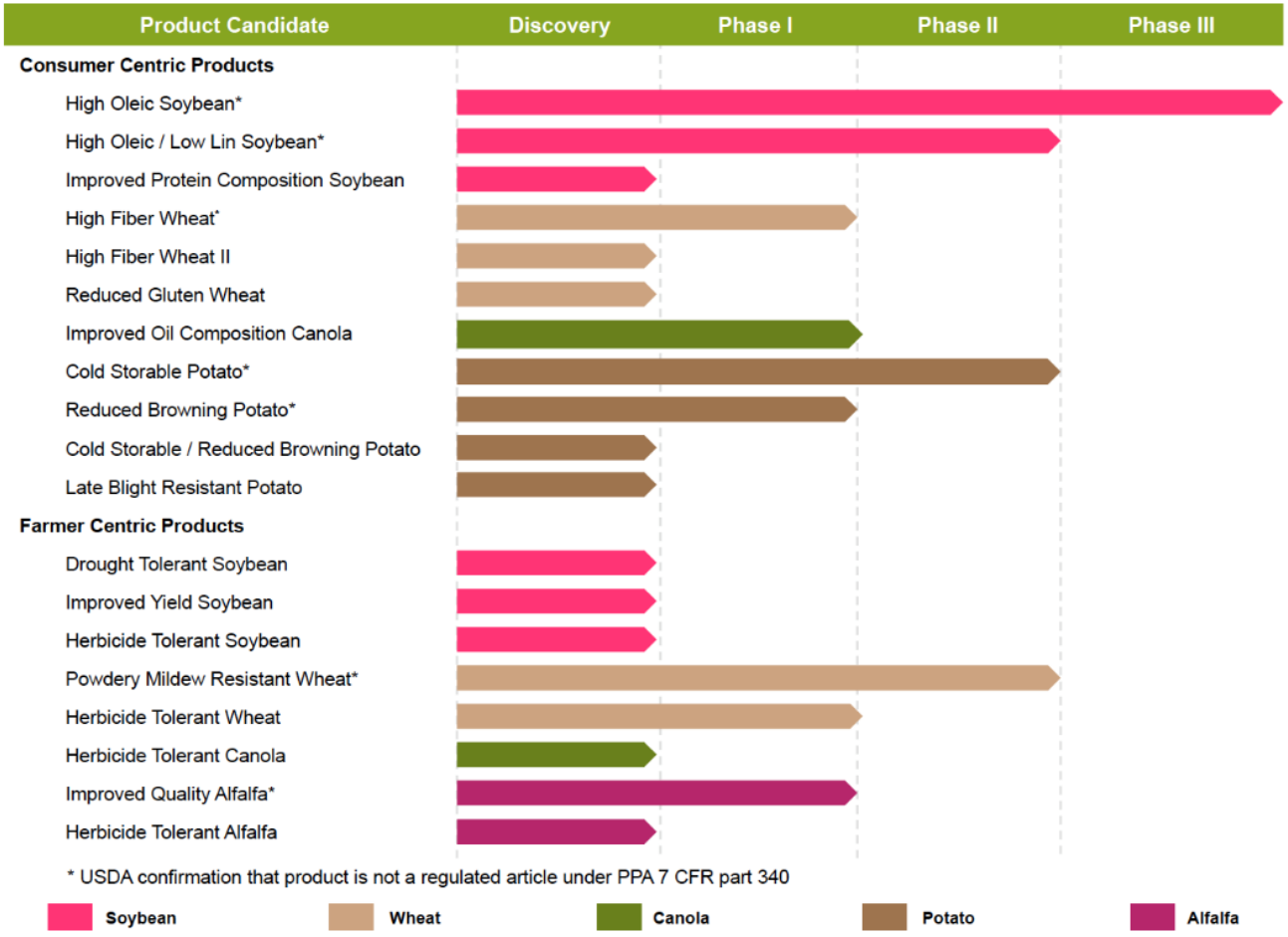
Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family

High oleic soybean



- 80% oleic acid
- 20% less saturated fat
- Zero trans fats

Huan et al. 2014 Plant Biotechnology Journal



http://www.calyxt.com/products/products-in-our-development-pipeline/

IP scenario for Ag CRISPR applications

NEWS / 10.18.17

DuPont Pioneer and Broad Institute Join Forces to Enable Democratic CRISPR Licensing in Agriculture

By David Cameron

New partnership provides nonexclusive licenses to CRISPR-Cas9 IP for commercial agricultural research

"The Broad Institute recently announced a significant cross-licensing effort for its CRISPR patents for agricultural applications. The agreement brings together the Broad, Monsanto, DuPont Pioneer, Caribou Biosciences, ERS Genomics, and Vilnius University"

Sherkow 2018

March 2018

Secretary Perdue Issues USDA Statement on Plant Breeding Innovation

Under its biotechnology regulations, **USDA does not currently regulate, or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques** as long as they are developed without the use of a plant pest as the donor or vector and they are not themselves plant pests. This can include plant varieties with the following changes:

Deletions: the change to the plant is solely a genetic deletion of any size.

Single base pair substitutions: the change to the plant is a single base pair substitution.

Insertions from compatible plant relatives: the change to the plant solely introduces nucleic acid sequences from a compatible relative that could otherwise cross with the recipient organism and produce viable progeny through traditional breeding.

Complete Null Segregants: off-spring of a genetically engineered plant that does not retain the change of its parent.

Regulated Article Letters of Inquiry

Data Updated: November 29 2018

Print

https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/Regulated_Article_Letters_of_Inquiry

Show 25 entries

Date	Institution	Description
11/29/2018	Lygos, Inc.	GE Pichia kudriavzevii modified for Industrial Applications
11/8/2018	Arbiom, Inc.	GE Trichoderma reesei modified for Industrial Applications
10/22/2018	Suntory Flowers Limited	Importation of Cut Flowers of GE Chrysanthemum
10/15/2018	BASF Enzymes LLC	GE Trichoderma reesei modified for Industrial Applications
9/27/2018	Yield10 Bioscience	Genome Edited Camelina Lines Developed with CRISPR/Cas technology
8/6/2018	Illinois State University	Genome Edited Pennycress Lines Developed with CRISPR/Cas technology
7/12/2018	Iowa State University	Genome Edited Maize Developed with CRISPR/Cas technology
5/18/2018	University of Georgia	Soybean Engineered for Transposon Mutagenesis that uses Trans-acting siRNA
5/18/2018	University of Georgia	Soybean Engineered for Transposon Mutagenesis
5/14/2018	University of Florida	Genome Edited Tomato Developed with CRISPR/Cas Technology
3/30/2018	TAXA Biotechnologies, Inc.	Fragrant Moss Developed Without Plant Pest Components
3/20/2018	Calyxt, Inc.	Nutritionally-Enhanced Wheat Developed by TALEN Technology

4/18/2016	DuPont Pioneer	Waxy Corn Developed by CRISPR-Cas Technology
4/13/2016	Penn State	CRISPR-edited Mushroom

5/20/2015	Cellectis Plant Sciences	FAD3KO Soybean
5/18/2015	Benson Hill Biosystems, Inc.	BHB Hi-Yield Maize
5/5/2015	Cellectis Plant Sciences	FAD2KO Soybean Without Materials from Plant Pest Sequences

USDA-APHIS "Am I regulated?" letters of inquiry

Gene-edited crops will generally show up here before being released onto the market

Proposed APHIS rule

Public comments through August 5, 2019

Proposed rule largely follows the March 2018 statement:

Gene edited lines will not be subject to regulatory review if they fall under one of the defined exceptions

Under proposed § 340.1(b)(1) through (4), modified GE plants would not be regulated or subject to a regulatory status review in accordance with § 340.4, if:

- The genetic modification is solely a deletion of any size; or
- The genetic modification is a single base pair substitution; or
- The genetic modification is solely introducing nucleic acid sequences from within the plant's natural gene pool or from editing nucleic acid sequences in a plant to correspond to a sequence known to occur in that plant's natural gene pool; or
- The plant is an offspring of a GE plant and does not retain the genetic modification in the GE plant parent.

NATIONAL ARCHIVES

FEDERAL REGISTER

The Daily Journal of the United States Government

PR Proposed Rule

Movement of Certain Genetically Engineered Organisms

A Proposed Rule by the Animal and Plant Health Inspection Service on 06/06/2019

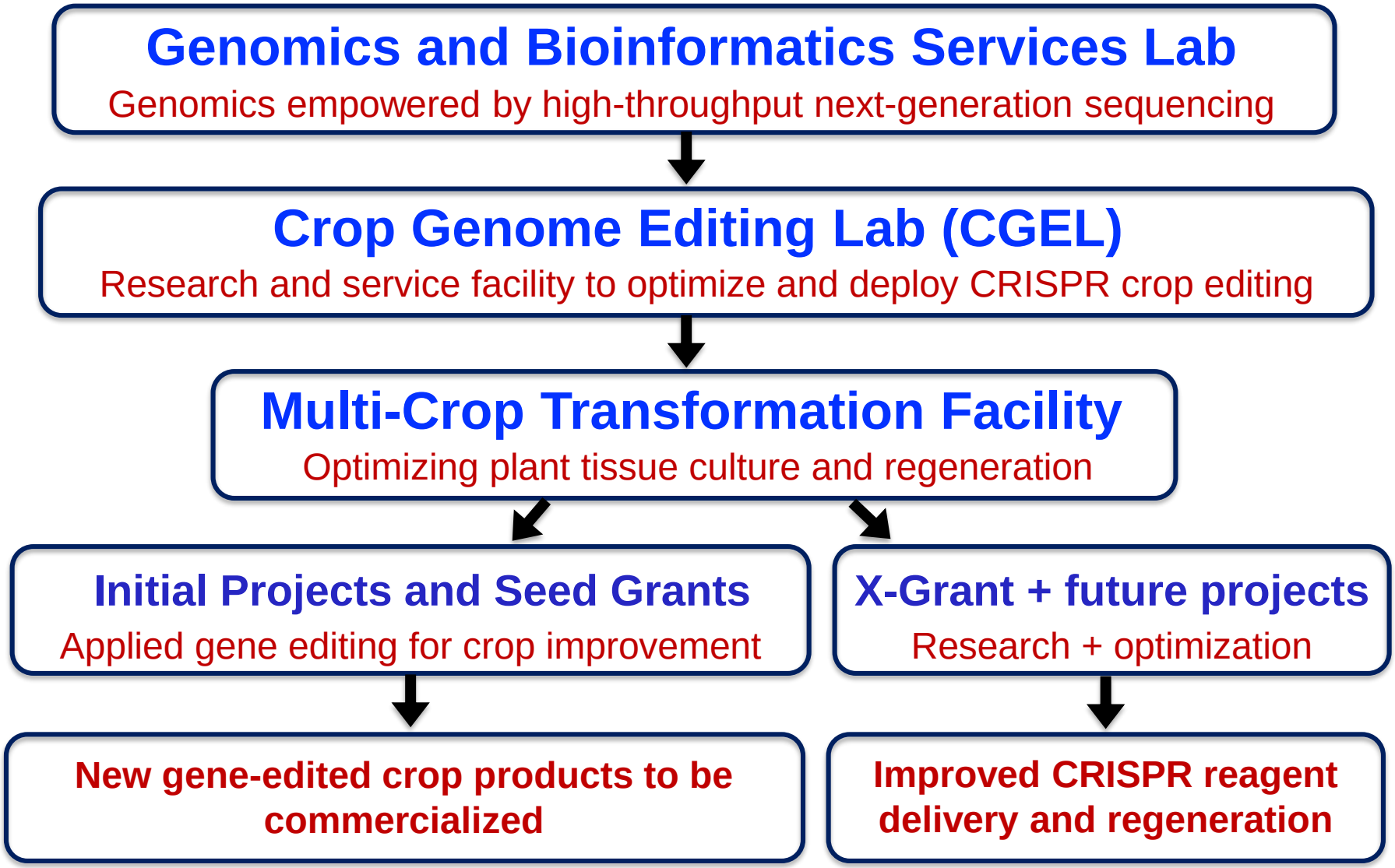
This document has a comment period that ends in 17 days. (08/05/2019)

SUBMIT A FORMAL COMMENT

Read the 736 public comments

https://www.federalregister.gov/documents/2019/06/06/2019-11704/movement-of-certain-genetically-engineered-organisms

Genomics, Gene Editing, and Transformation pipeline at Texas A&M University



Texas A&M AgriLife Research
Crop Genome Editing Lab (CGEL)

- New facility recently set up on the Texas A&M campus for CRISPR-based genome editing in crops
- Will provide research, service, and training functions to optimize protocols, set up a high throughput gene editing pipeline, and enable development of gene-edited products
- Rice is being used to optimize more efficient genome editing approaches, including non-transgenic approaches

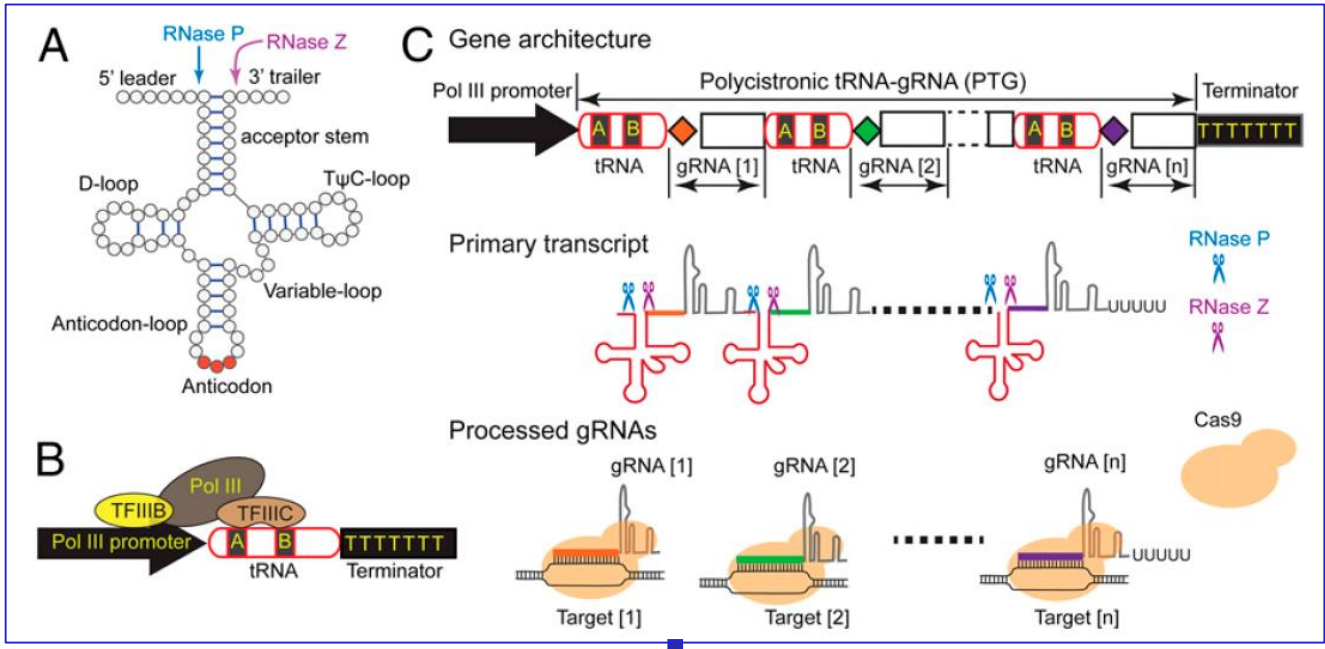
https://agrilife.org/cgel/

AgriLife Research Gene Editing Seed Grants

13 Seed Grant projects:

- Target gene sequence analysis
- gRNA design
- Transformation vector construct development
- Provide to transformation lab
- Assist in screening edited progeny to confirm mutations
- Rice, wheat, sorghum, cotton, potato, and melon

CGEL using a polycistronic tRNA-gRNA approach for multiplexed editing:



Multi-Crop Transformation Facility for Agrobacterium transformation, tissue culture, and regeneration

Texas A&M University President's Excellence Fund

X-Grant Program: "CRISPR Crops Initiative"

Interdisciplinary project with 17 members

Charlie Johnson
Aniruddha Datta

Byung-Jun Yoon
Xiaoning Qian
Yang Shen

Genomics and Bioinformatics Service

Texas A&M AgriLife Research

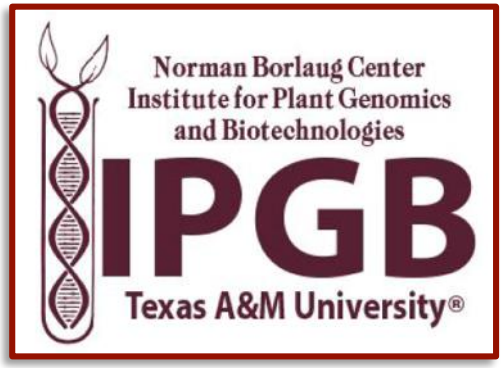
Charlie Johnson

Crop Genome Editing Lab

Michael Thomson

John Mullet
Joseph Awika
David Stelly

Endang Septiningsih
Sakiko Okumoto
Jean-Philippe Pellois



Libo Shan
Ping He
Keerti Rathore

Multi-Crop Transformation Facility

Marco Molina
Mayra Molina

X-Grant Program: “CRISPR Crops Initiative”

Goal: to test innovative ideas enabling a high-throughput CRISPR gene editing workflow that will spur the next generation of breakthroughs in crop improvement

Analyze the wealth of genomics data to identify key genes to modify

Test high-risk, high-reward technologies to increase CRISPR efficiency 100-fold

Apply these new approaches and technologies to high-impact pilot studies

Conclusions

- CRISPR/Cas9 has tremendous potential to make a massive impact in crop improvement
  - **Leverage knowledge of genetics/genomics to better understand key genes and introduce novel traits**
  - **Make novel genetic diversity more accessible and readily usable**
  - **Empower plant breeders to precisely combine desired traits**
- IP situation and government regulations for genome editing are in flux, but becoming more clear
- Consumer acceptance will be essential moving forward

Thank You!