7th Seed Congress of the Americas

Promoting Seed Business in the Americas

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Gene editing
Uses, benefits & opportunities: an introduction to the topic

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SAA
Seed Association of the Americas

Technological obsolescence

When a **technical** product or service is no longer needed or wanted even though it could still be in working order. **Technological obsolescence** generally occurs when a new product has been created to replace an older version.

http://www.businessdictionary.com

Low-Tech era:

Medium Tech Era:
Non-targeted DNA transfer.

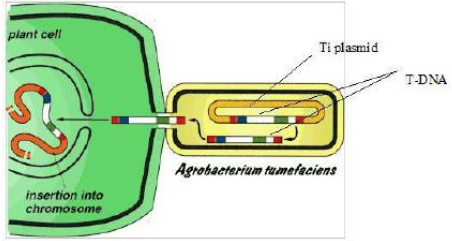
High Tech Era: Targeted Genome Changes.

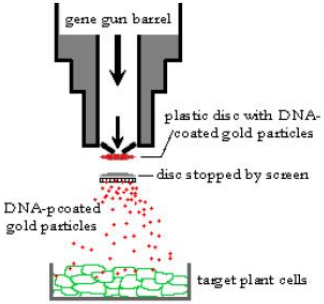
1980 1990 2010 2012



Cross compatable plants.
Select based on phenotype.

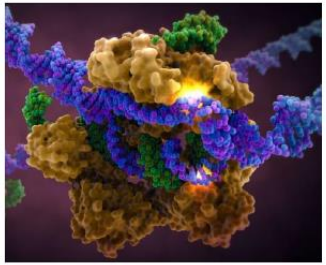
Later…Induce random mutations

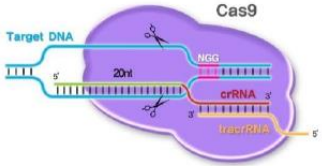






CRISPR-Cas as a targeted DNA cleaving enzyme.





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Genome Editing:

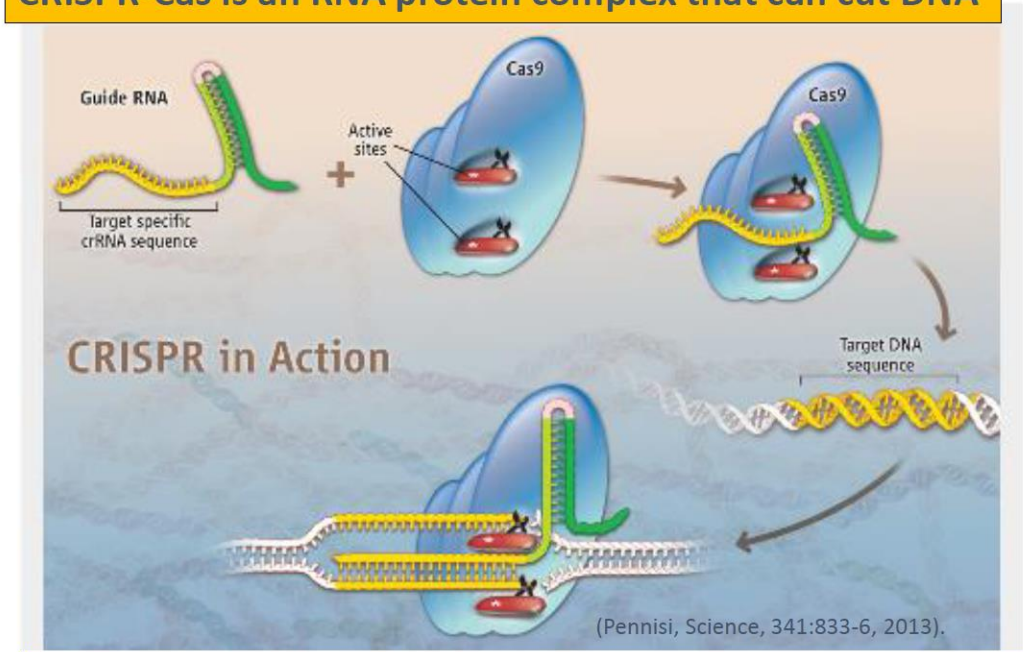
- What is Genome Editing?
  - The Ability to re-write a genome.
  - Add, Delete, or Change specific DNA sequences.
- In Editing we make changes WHERE we want.
  - Inserted genes in "Transgenics" (1990s-2010s) were not targeted to a specific location.



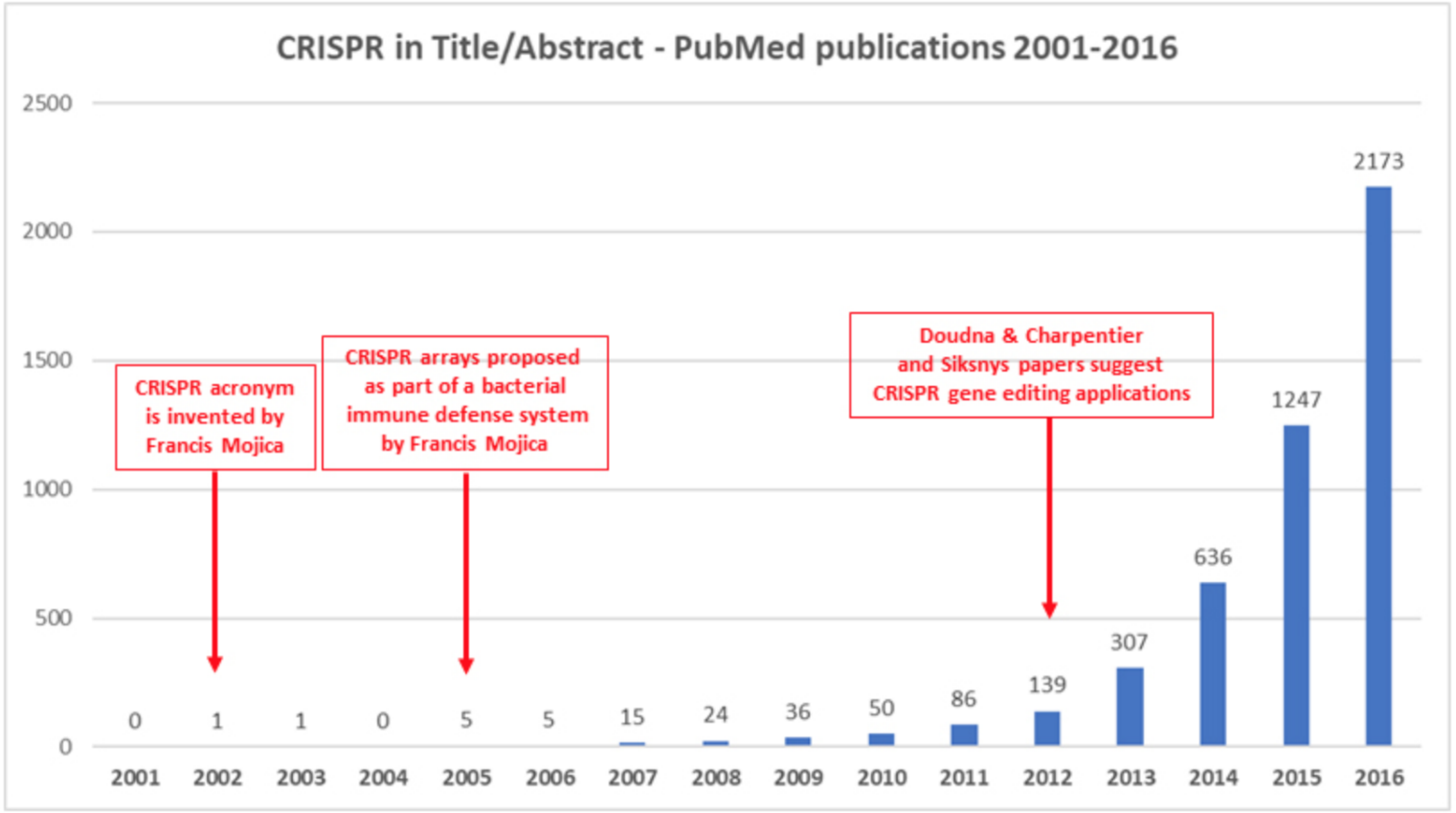
- Genome Editing is now, easy, efficient, cheap and accessible to anyone.....because of the CRISPR/Cas enzyme.

Clustered Regularly Interspaced Short Palindromic Repeats.

CRISPR-Cas is an RNA protein complex that can cut DNA



CRISPR is like a “tunable” molecular scissors for DNA.
To direct CRISPR to a ‘new’ DNA site, we simply provide it with a new Guide RNA.



(by Lluís Montoliu ©)

Gene Editing Research

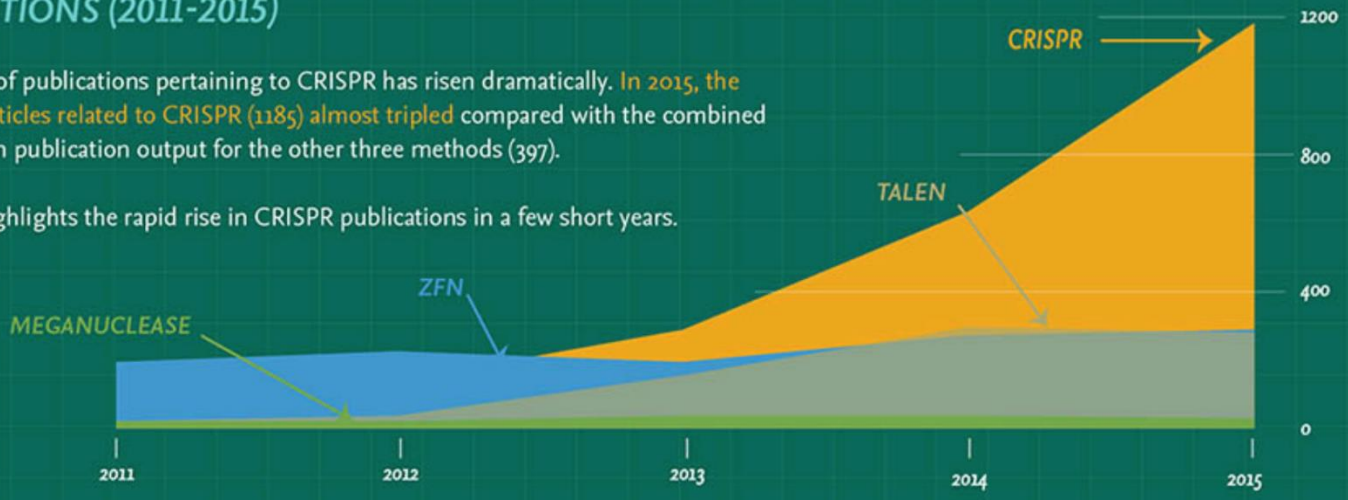
SciVal

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PUBLICATIONS (2011-2015)

The number of publications pertaining to CRISPR has risen dramatically. In 2015, the number of articles related to CRISPR (1185) almost tripled compared with the combined growth rate in publication output for the other three methods (397).

The graph highlights the rapid rise in CRISPR publications in a few short years.



Using Scopus data, analyzed in SciVal (2011-2015)

Why is there so much interest in the CRISPR nuclease?

Flexibility

Targetable ("tunable") DNA cutting enzyme.

Efficiency

Very efficient at cutting DNA in cells (some experiments >50%)

Cost

Very inexpensive to reprogram CRISPR

(~$20 to make a guide RNA)

Ease of Use

Simple to re-engineer CRISPR to cut the site you want.

Guide RNA design may take 1 hour….or less.

Order Guide RNA sequence from vendors. Receive in ~2 days.

Applications of Gene Editing in plant Breeding.
What you can do with Genome Editing Techniques?

1. Targeted Gene Knockouts
2. Single nucleotide change
3. Targeted Gene (DNA) Replacement
4. Targeted DNA deletion

Targeted Nuclease mediated Gene knock out examples

NATURE BIOTECHNOLOGY | RESEARCH | LETTER

日本語要約

Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew

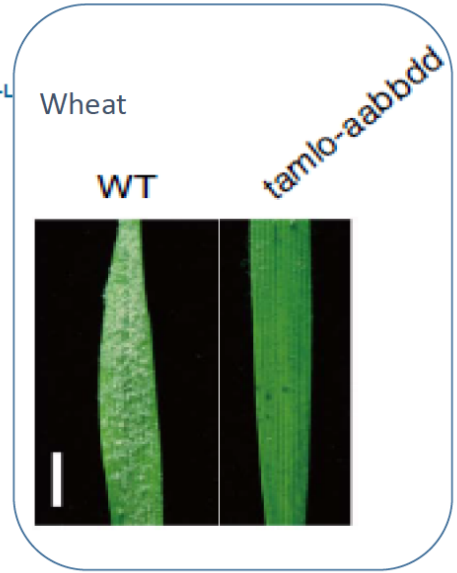
Yanpeng Wang, Xi Cheng, Qiwei Shan, Yi Zhang, Jinxing Liu, Caixia Gao & Jin-L

Affiliations | Contributions | Corresponding authors

"Knocking out" Susceptibility genes in the plant…. can lead to resistance.

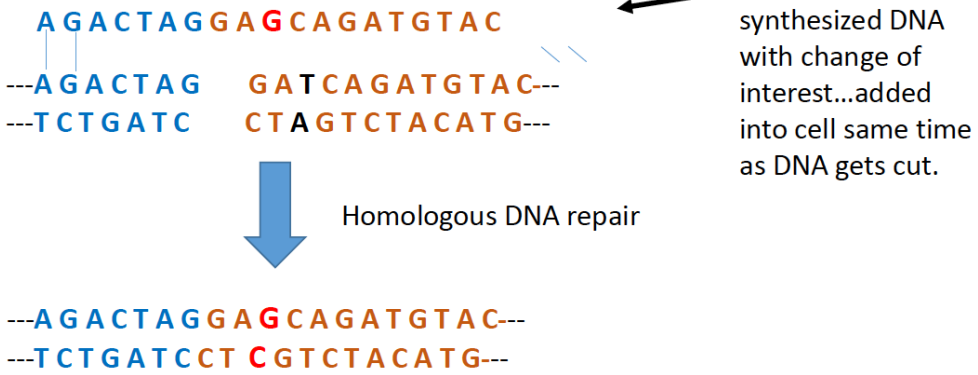
Susceptibility gene = a plant gene that is REQUIRED for a pathogen to replicate or induce disease.

Simultaneous KO of multiple gene copies is enabling.



Genome Editing: single nt change in DNA

- 2. Make a *single* nucleotide change in a targeted DNA sequence.
- Introduce into plant cell a site specific nuclease AND a homologous template that has ONE nucleotide change.
- Homology Directed Repair (HDR) repair pathway will 'fix' break with provided template, introducing targeted change into plant DNA.



Piece of synthesized DNA with change of interest…added into cell same time as DNA gets cut.

Changing a single targeted nucleotide in Maize generated herbicide resistant Maize

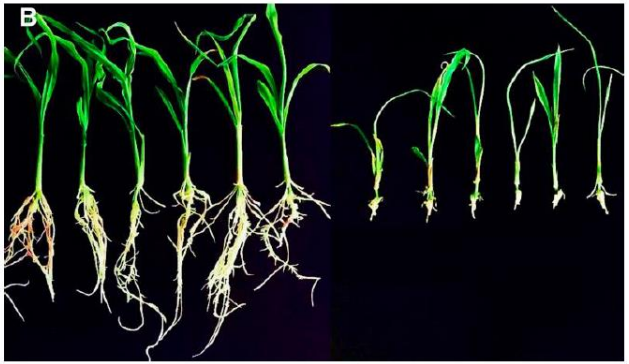
Herbicide R mutant. WT



Maize: single nt Gene editing of ALS2 (Chlorosulfan R)

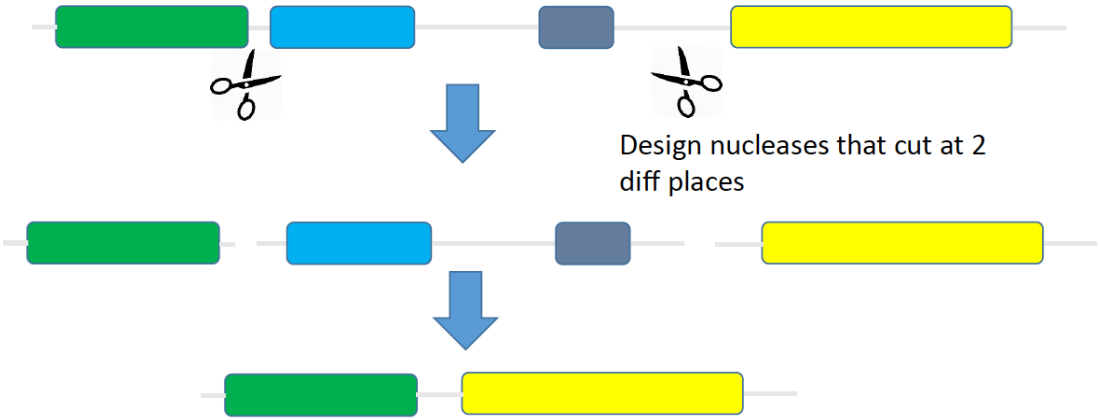
ALS: acetolactate synthase

Herbicide R mutant. WT



T1 generation embryos germinated on media with chlorsulfuron (100 µg L−1) 14 d after germination.

Genome Editing: Targeted Genome Deletions



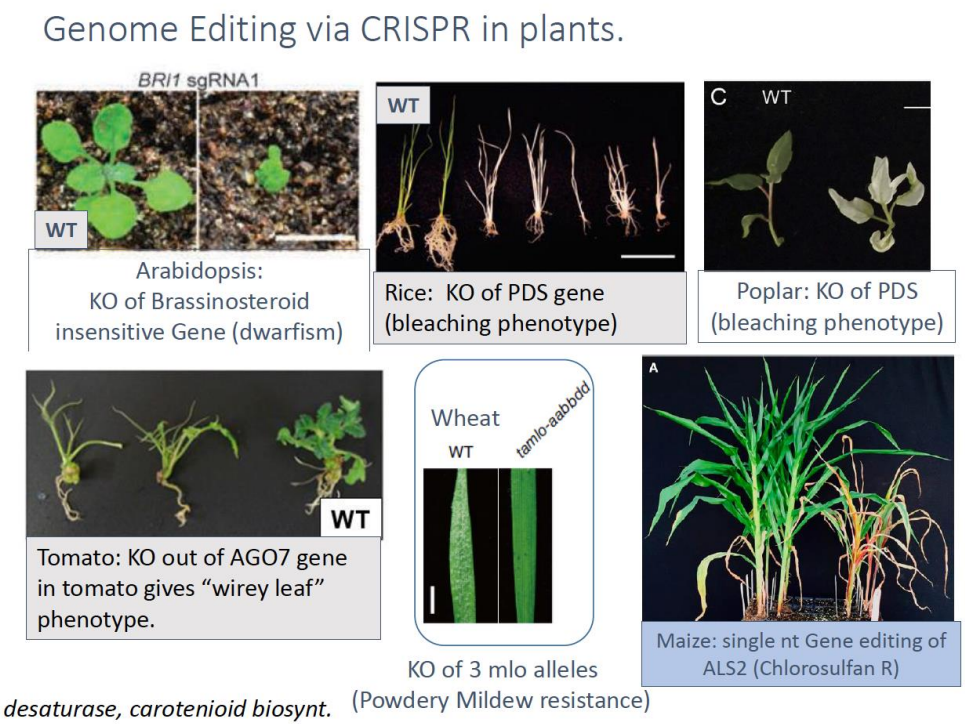
Design nucleases that cut at 2 diff places

Small (less than 100 bp) to large (greater than 10,000 bp) of DNA has been deleted with this approach.



Tomato: KO out of AGO7 gene in tomato gives "wirey leaf" phenotype. *Argonaute Prot*

Genome Editing via CRISPR in plants



PDS: phytoene desaturase, carotenioid biosynt.

Key Issues Impacting use of Gene Editing in plant Breeding

1. Knowledge (what changes to make).
2. Technical Expertise.
3. Access to Technology.
4. Regulatory environment.



Key Issues….

1. Knowledge (this is different than just DNA sequence information)

   Understand where Genes start and stop.

   On/off Switches (what turns this gene on or off?)

   Function of a gene… what part of the plants biology does this effect?

   How many genes are involved in a trait?

   Ex: plants often have multiple copies of a gene…

   Built in 'backup system' in case on gene gets broken…

   The more you know….the more you can potentially do.

   Our Knowledge is incomplete.

Key Issues

2. Technical Expertise

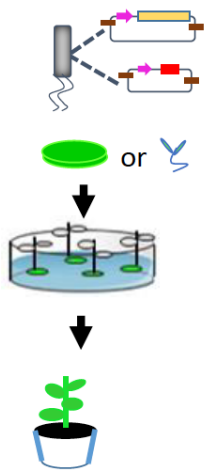
To deliver active CRISPR DNA cutting enzymes to plant cells.

To recover plants with modified (edited) genomes.

Tissue culture steps are needed to regenerate whole plants after Genome Editing by CRISPR.

Regenerating plants after using CRISPR for genome editing is a **bottleneck**.

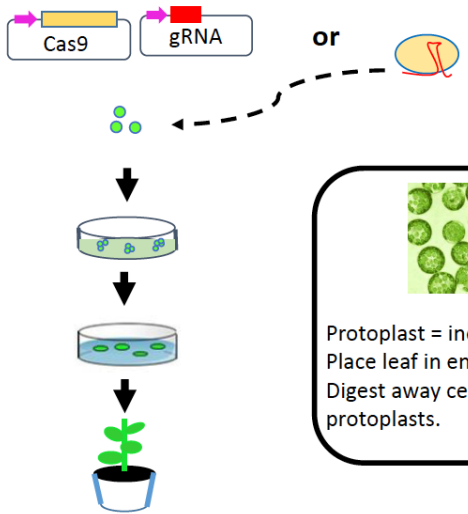
Agrobacterium
T-DNAs

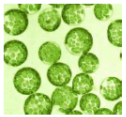


~6 months
Least-complicated TC.
Plants transgenic for Cas gene.
Transgene can be segregated away.

DNAs for producing Cas9 protein and guide RNA

Cas Protein-RNA complex





Protoplast = individual plant cells.
Place leaf in enzyme solution.
Digest away cell walls to make protoplasts.

~9 months
More-complicated TC.
Plants not transgenic.
Fewer species than leaf-disc transformation.

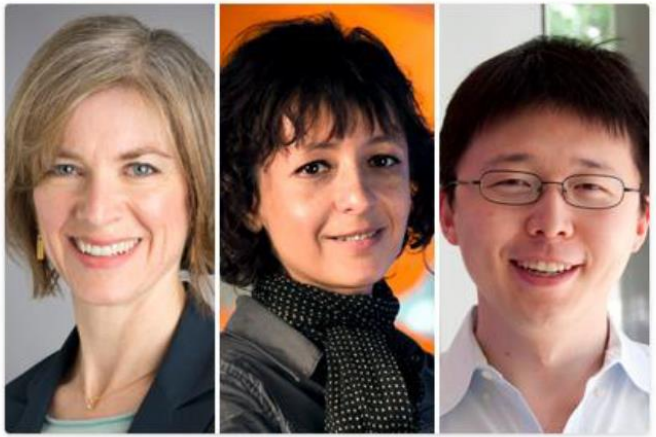
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Key Issues….

- 3. Access to Technology

- Who owns CRISPR-Cas?
- Broad Institute has awarded Patents.
- UC-Berkeley has patents filed, and an interference
- Figuring out who to license from is an issue…



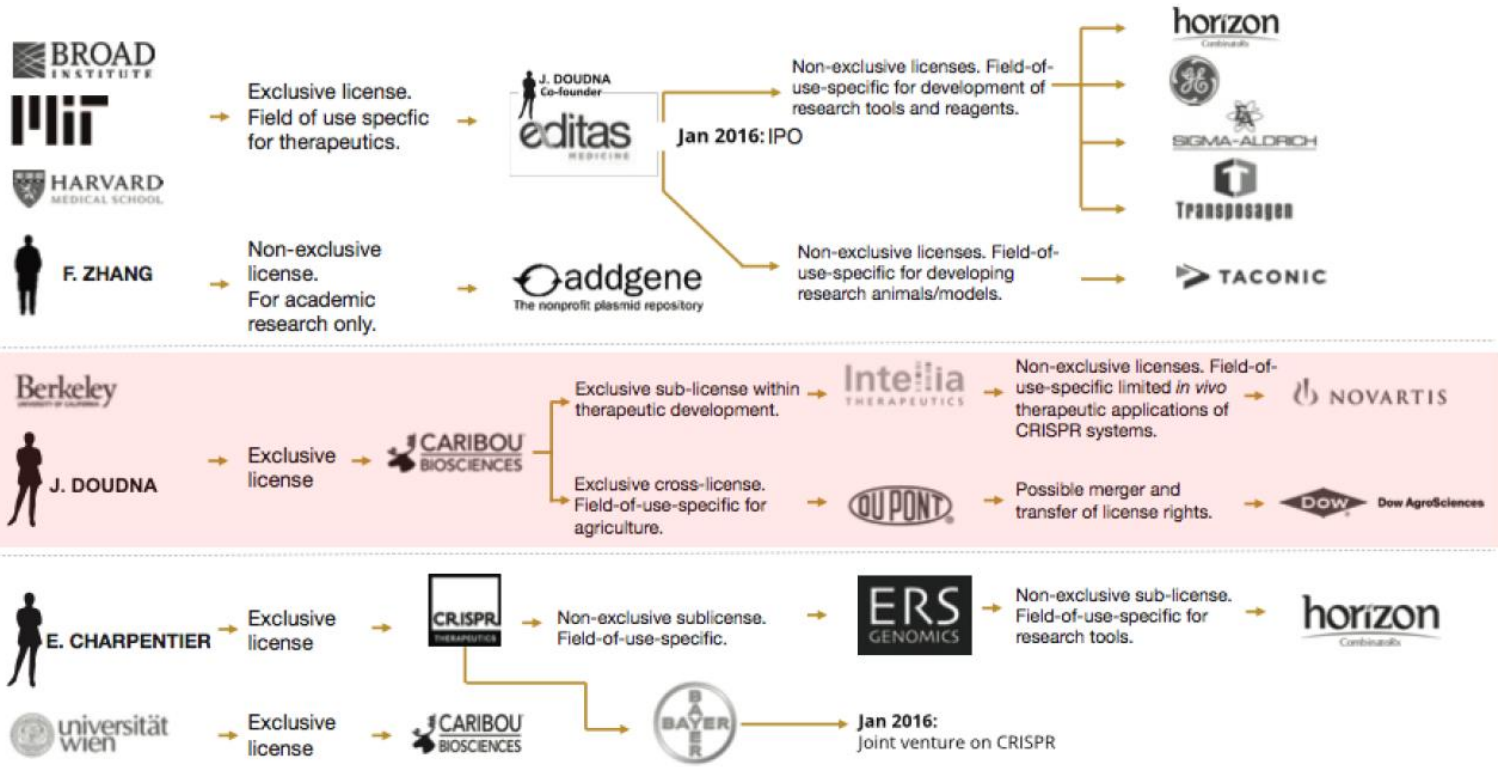
Doudna, Charpentier, and Zhang

The Scientist » News & Opinion » News Analysis

Who Owns CRISPR?

With one US patent awarded and many other applications under consideration for the popular genome-editing technology, companies are adopting multiple strategies to navigate the complex intellectual property landscape.

Early-phase CRISPR–Cas9 platform patent control and access



Key issues…

- 4. Regulatory Environment.

- Regulations for biotech ag products were originally drafted in early 1990s.

- 1990s Technology:
- Ability to move DNA into plants.
- DNA gets inserted at random locations
  - Best use is for moving entire genes into plants.

- Today:
- Ability to change just one nucleotide at a time
- Ability to make targeted changes.

Key issues…

- 4. Regulatory Environment.
- With Genome Editing using CRISPR…you can make a SINGLE nucleotide change.
- How should (will) this be regulated?
- Consider this: “Mutagenic breeding” is not-regulated.
- Mutagenic breeding:
- treat seeds with Chemicals or radiation.
- Treatment creates 100s or 1000s of random genetic mutations.
- Breeder selects plant with phenotype of interest; uses it in breeding program.
- NOT Regulated….over 3000 varieties generated with this strategy.
- Q. If you make a single, targeted genetic change with CRISPR…should this be regulated differently than ‘mutagenic

Genetically engineered crops that fly under the US regulatory radar

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14 EU countries call for 'unified approach' to gene editing in plants

By Gerardo Fortuna | EURACTIV.com



On 14 May, the EU ministers for Agriculture gathered in Brussels for the Agrifish Council. [EUROPEAN UNION]

Andriukaitis: New plant breeding techniques need new regulatory framework

By Sarantis Michalopoulos | EURACTIV.com



Andriukaitis insisted that Europe should listen to science otherwise "it has no chance to have sustainable agriculture and preserve biodiversity". [Shutterstock]

https://en.euractiv.eu/wp-content/uploads/sites/2/special-report/EURACTIV-Special-Report-The-EU-future-of-new-plant-breeding-techniques.pdf

UCDAVIS
Chile Life Sciences Innovation Center
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Gene editing: Uses, benefits & opportunities: an introduction to the topic

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