Introduction of CRISPR technology
What could it mean for food?

Dr. Wilbert Sybesma

CRISPR-Based Technologies and the Future of Food Science

- CRISPR principles
- SSDR-CRISPR in lactic acid bacteria
- Examples relevant for agriculture and food
- For discussion:
  - *How can CRISPR be used for next-generation food products with enhanced quality and health promoting functionalities?*

Published in final edited form as:
Biotechnol Bioeng. 2016 May ; 113(5): 930–943. doi:10.1002/bit.25851.

Current and future prospects for CRISPR-based tools in bacteria

Michelle L. Luo, **Ryan T. Leenay**, and **Chase L. Beisel***
Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695, USA

ELSEVIER
Current Opinion in Biotechnology
Volume 37, February 2016, Pages 61–68
Food biotechnology • Plant biotechnology

Exploiting CRISPR–Cas immune systems for genome editing in bacteria

Rodolphe Barrangou[1], Jan-Peter van Pijkeren[2]
Show more

http://dx.doi.org/10.1016/j.copbio.2015.10.003

Journal of Food Science
A Publication of the Institute of Food Technologists
Explore this journal >

R: Concise Reviews in Food Science

CRISPR-Based Technologies and the Future of Food Science

Kurt Selle, Rodolphe Barrangou

First published: 7 October 2015 Full publication history
DOI: 10.1111/1750-3841.13094 View/save citation

van Pijkeren and Britton *Microbial Cell Factories* 2014, **13**(Suppl 1):S10
http://www.microbialcellfactories.com/content/13/S1/S10
MICROBIAL CELL FACTORIES

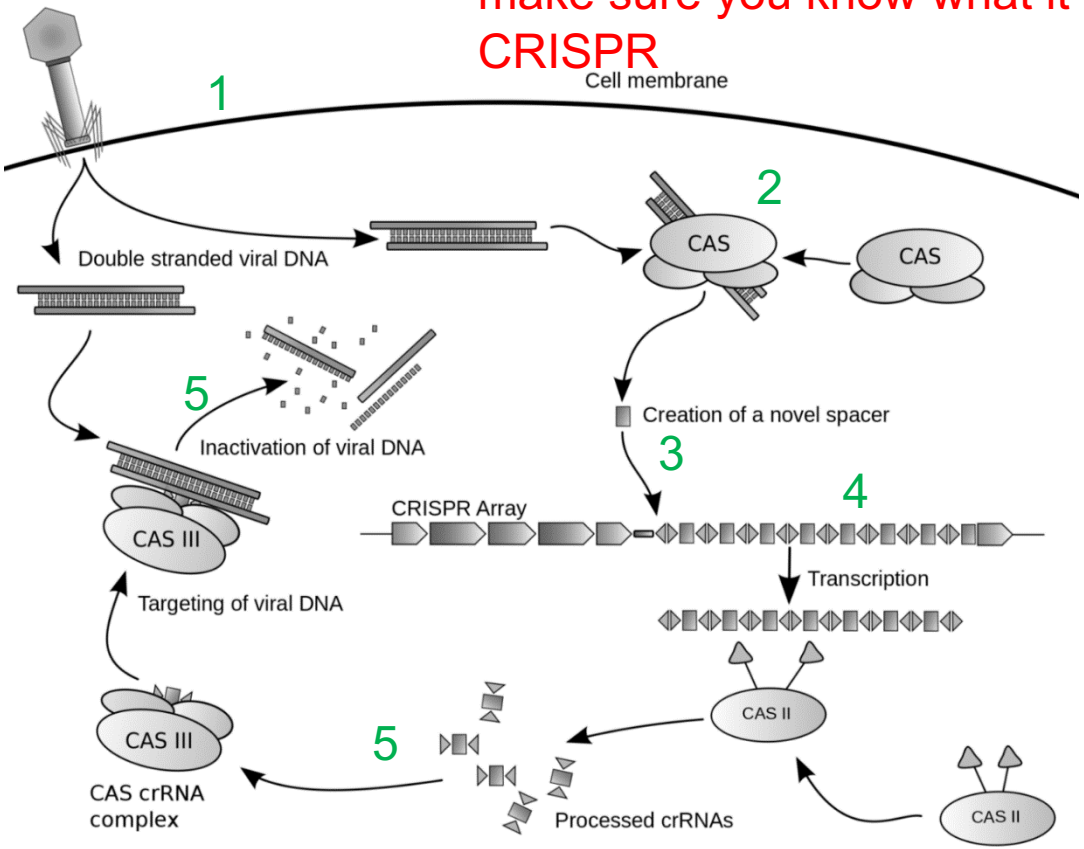
PROCEEDINGS Open Access

Precision genome engineering in lactic acid bacteria

Jan Peter van Pijkeren[1], Robert A Britton[2*]

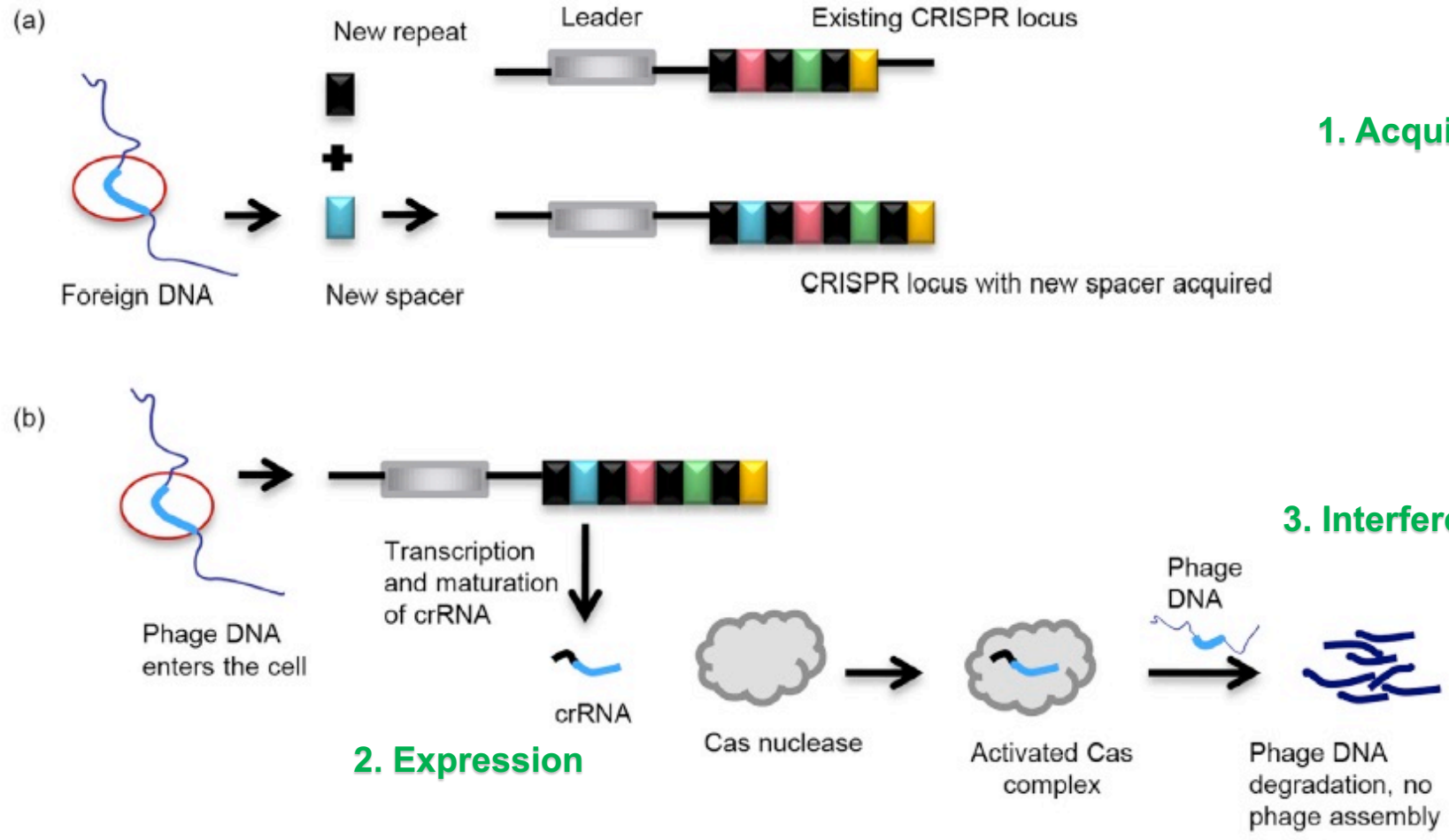
CRISPR - principles

No Need to Understand CRISPR mechanism by heart, but make sure you know what it is and what you can do with CRISPR

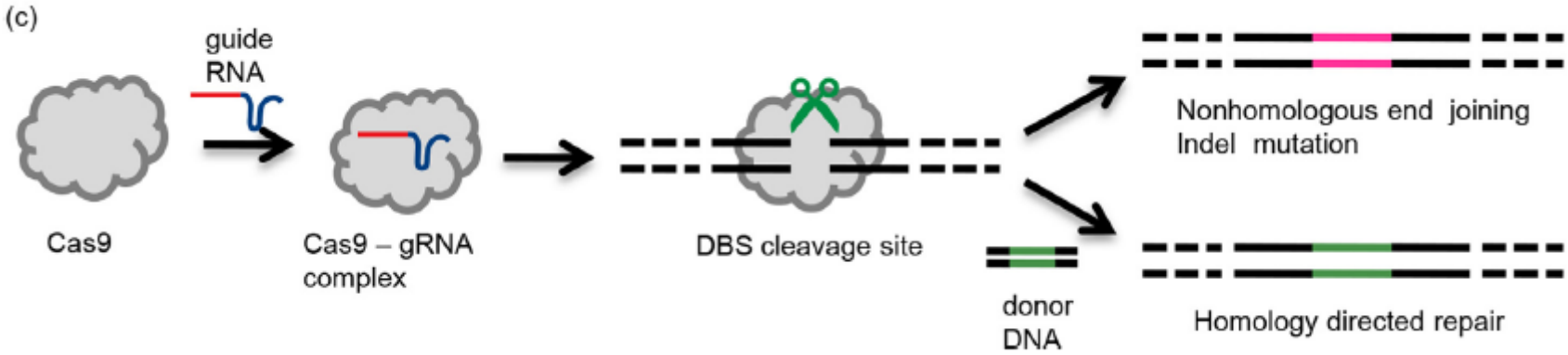


- 1,2,3: Short segments of foreign (bacteriophage) DNA, spacers, are incorporated into the genome between CRISPR repeats
- 4: They serve as a 'memory' of past exposures.
- 5,6: CRISPR spacers are then used to recognize and silence exogenous genetic elements in a manner analogous to RNAi in eukaryotic organisms

CRISPR - principles



Genome editing by incorporating «guide RNA»



CRISPR-Cas9 as molecular scissor

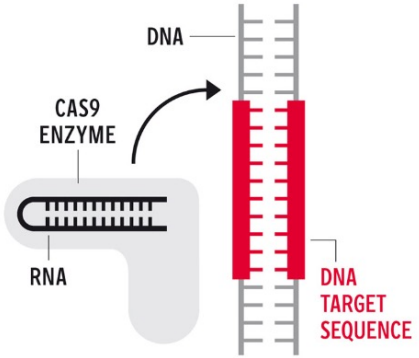
Clustered Regulatory Interspaced Short Palindromic Repeat associated with Cas9 nuclease

A BRAVE NEW WORLD OF GENOME EDITING

How the Crispr system derived from bacteria works on human cells to correct genetic defects

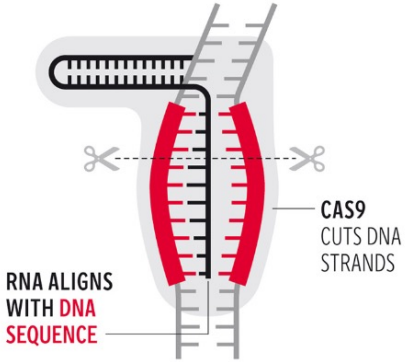
1

An RNA "guide" molecule can be **programmed to match any unique DNA sequence** found in the human genome



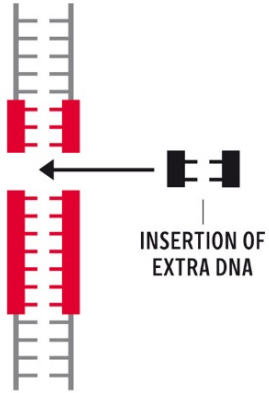
2

A special enzyme, called **CAS9, can be attached to the RNA guide**. Its job is to find the target sequence of DNA



3

The RNA aligns with the target DNA sequence and the **CAS9 attaches and cuts both strands of the DNA double helix**



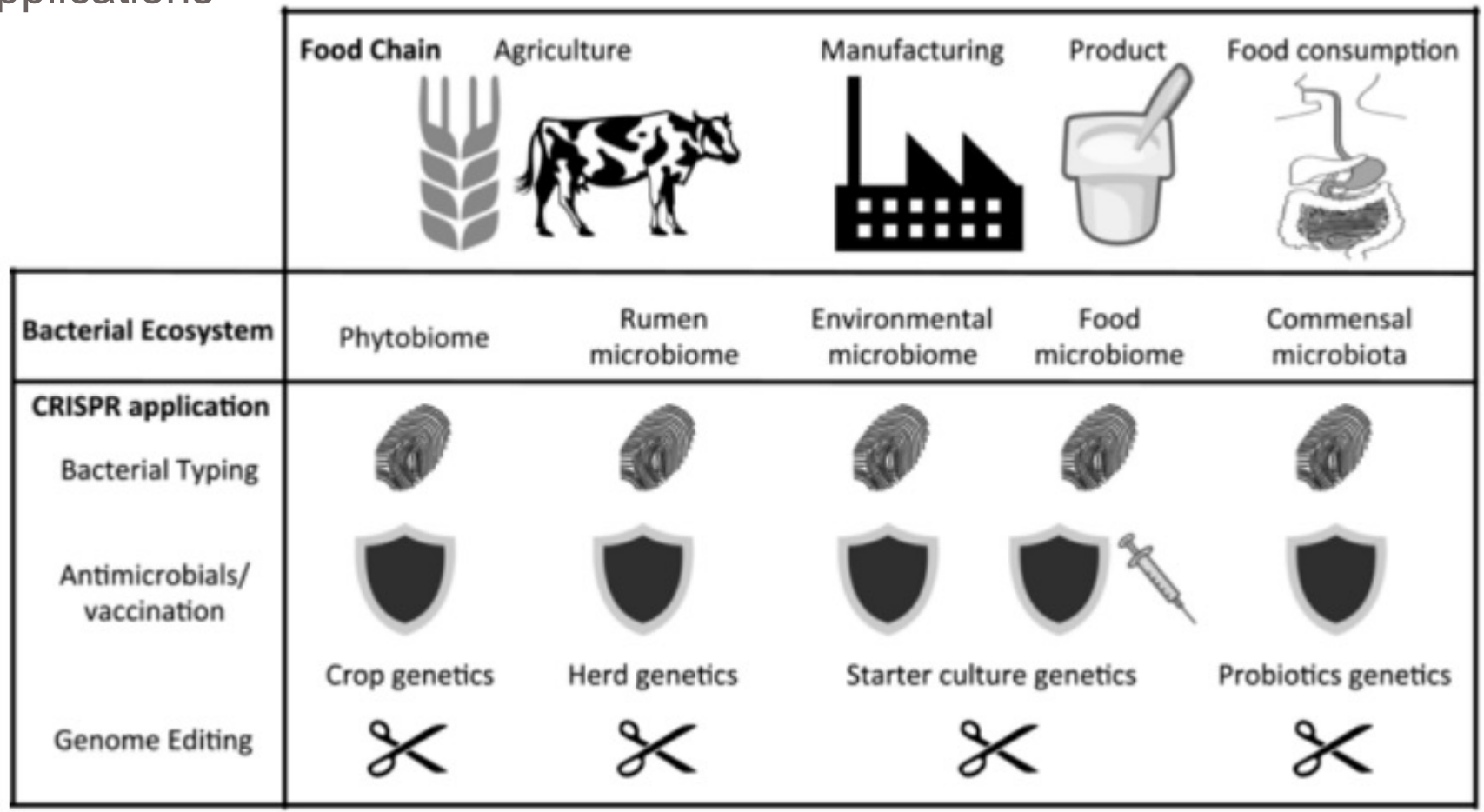
4

The DNA cuts can be **amended with an extra DNA insertion** (above), or a deletion of defective DNA

SOURCE: UC BERKELEY

Final product is a organism that contains a user-defined base-change(s) in its genome, which genetically cannot be distinguished from a natural-mutant isolate

Applications

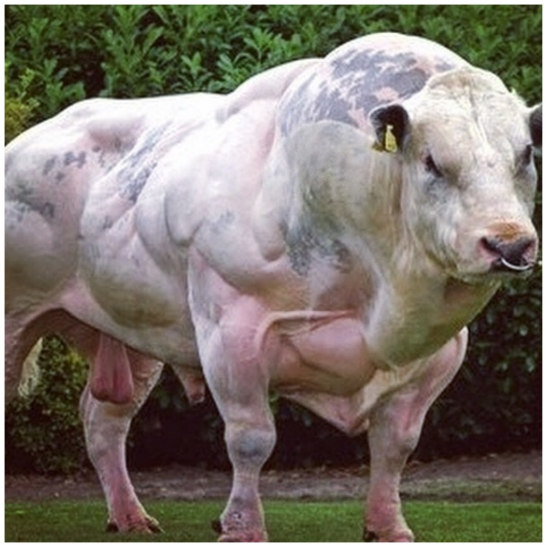


Double-muscled pigs are made by disrupting, or editing, a single gene

A team of scientist from South Korea and China developed muscly pigs
The myostatin gene (MSTN) functions as a negative regulators of muscle growth. Mutations of the myostatin MSTN gene induce proliferation of the muscle cells creating abnormal bulk of muscle fibers. CRISPR-Cas9 was used to edit the MSTN gene, inducing segment deletions/inversions, in porcine embryo fibroblasts. Via SCNT, cloned pigs with homozygote mutations were generated. Similarly to Belgian Blue and Piedmontese cattle breeds, which harbor natural mutations in MSTN gene, muscly pigs were engineered for meat production.

- **Super-muscly pigs created by small genetic tweak**

Nature, 523, 2 July 2015



Belgian Blue bull

Muscly pigs could become the first GM animal approved for human consumption

Gene edited mushroom and waxy maize got USDA approval

PENN STATE | NEWS

Home Research Academics Impact Campus Life Athletics Administration Arts and Entertainment

An anti-browning mushroom developed by plant pathologist Yinong Yang using CRISPR-Cas9 gene-editing technology will have a longer shelf life and resist blemishes from handling and mechanical harvesting. USDA has ruled that the mushroom is not subject to the agency's regulatory process for GMOs.

Image: Yang Lab

Gene-edited mushroom created by Penn State researcher is changing GMO dialogue

© Dinodia Photos / Alamy Stock Photo

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

Gene editing on the farm - Genome editing plays an increasing role in the improvement of animal traits for agriculture

- Production of hornless dairy cattle from genome edited cell lines
  Nature Biotechnology, 2016, **34**, 5, 479-481

recombinetics

Photograph of Spotigy at 2 months of age, so named after the black spots where horn buds would have developed.

Photograph of Buri (left) and Spotigy at 2 months of age

Gene editing to engineer pigs that are more resilient to a deadly virus infection

- **Pig's genetic code altered in bid to tackle deadly virus**

Norrie Russell, Univ. Edinburgh

Pigs reared at the University of Edinburgh's Roslin Institute have had individual letters of their genetic code modified to protect them against African swine fever.

In pigs, the susceptibility to the haemorrhagic Swine Fever Virus infection is associated with RELA gene. Swine Fever infection is a highly contagious disease that kills up to two-thirds of infected animals.
The African warthog (which belong to the same family as domestic pigs) tolerate the infection; its RELA gene sequence differs from that of domestic pig by only 3 times 3 nucleotides out of more than about 1600 (GenBank: FN999989.1).
At the Scotland's Roslin Institute, the RELA gene of embryo fibroblast cell lines of pigs were mutated by means of CRISPR in order to achieve the exact warthog RELA sequence. The cell lines were cloned by SCNT and after pregnancies, the born piglets were resistant to haemorrhagic Swine Fever Infection. Phacochère = warthog

ROSLIN

THE UNIVERSITY of EDINBURGH

Efficient Generation of Myostatin Mutations in Pigs Using the CRISPR/Cas9 System (Scientific Reports | 5:16623 | DOI: 10.1038/srep16623)

China ‘cloning factory’ to produce cattle, racehorses and pets

Chinese biotechnology firm Boyalife and South Korea's Sooam Biotech are setting up the world's biggest animal cloning factory in China, planning to churn out a million beef cattle a year (AFP Photo/Guillame Souvant)

BOYALIFE®
For Better Health

« simple » gene editing targets be for coffee and cacao

- **« Caffeine gene knock out » for caffeine free coffee**
  (small gene family associated with caffeine synthesis and a Japanese academic group has already showed that caffeine was reduced using antisense technology …. CRISPR may be a better approach)

- **Viral immunity in cacao** (develop immunity to Cacao Swollen Shoot Virus – CSSV; a growing issue for Cacao)

III Issues

a) Regulatory

b) Patents/Licensing for CRISPR in Plants

c) Consumer Acceptance

BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency's regulatory process — making it the first CRISPR-edited organism to receive a green light from the US government.

"The research community will be very happy with the news," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yinong Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (*Agaricus bisporus*) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a hand-

STUART MCCALL/GETTY

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

Nature 2016

The gene edited polled calves with a PP genotype can passed on the polled trait to the subsequent generations.

Polled Bull ♂	p	Horned Cow ♀ p	p
p	Pp Polled*	Pp Polled*	
p	Pp Polled*	Pp Polled*	

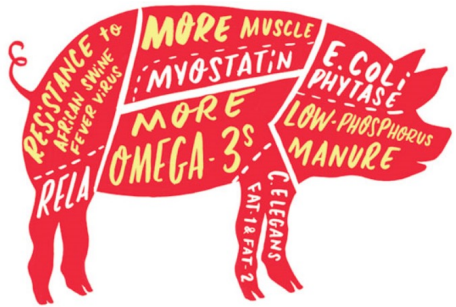
Mating a homozygous polled bull to homozygous horned females and the possible genotypes and phenotypes of the calves.

Genotype	Phenotype
PP	Polled
Pp	Polled (Scurs Possible*)
pp	Horned

From: Genetics of Horned, Polled and Scurred Cattle • www.eBEEF.org • 2015

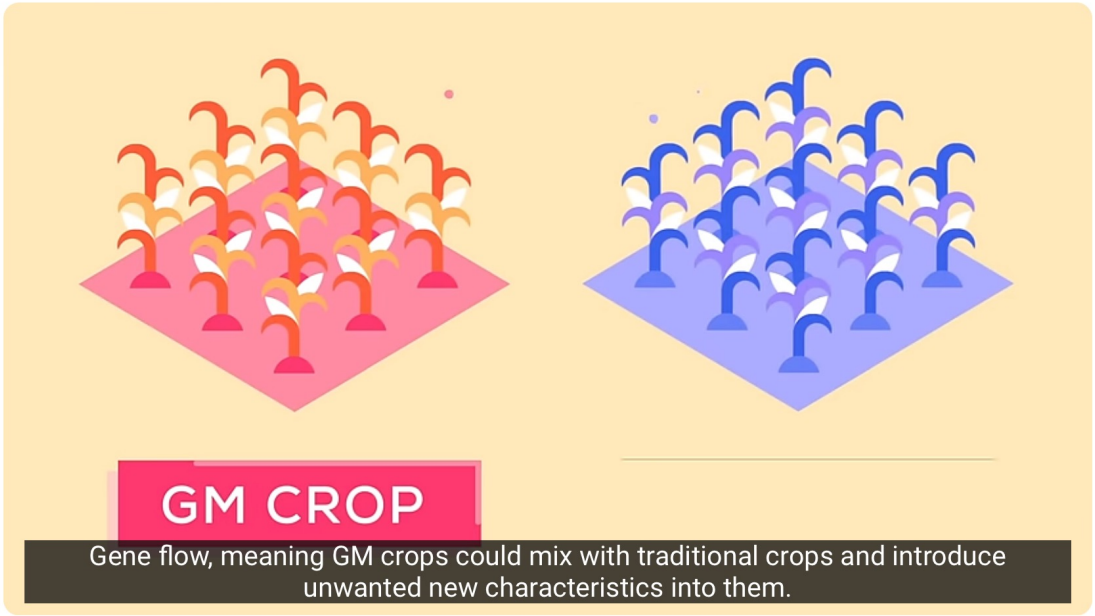
Conclusions

- Gene editing allows to “cut and paste” the genome
- As a technology, gene editing is:
  - Accurate
  - Rapid
  - Reliable
  - Cheap
- Gene editing impacts agriculture and food:
  - Gene editing in the fields ⇨ crops breeding activities (“CRISPR crops”)
  - Gene editing on the farm ⇨ livestock breeding activities
  - Geen editing for improvement of microorganisms
- Great impact of gene edited crops & livestock in the next 5 years (commercialization)
- Regulatory status not assessed yet and controversial



Illustrations from J. Holland, The Scientist, June 2014

https://www.youtube.com/watch?v=7TmcXYp8xu4



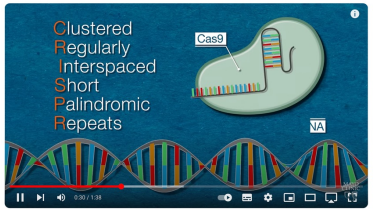
Are GMOs Good or Bad? Genetic Engineering & Our Food

https://www.youtube.com/watch?v=UKbrwPL3wXE 2 min.

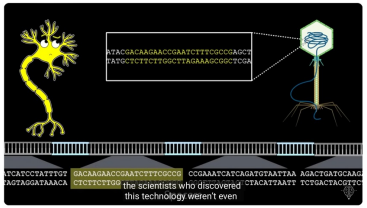
https://www.youtube.com/watch?v=ANehpGhbuF4 10 min.

https://www.youtube.com/watch?v=MnYppmstxIs 7 min.

Please check one of these movies to understand CRISPR.
(I think the 7 minutes movie is best, as it also explains the origin of CRISPR)



CRISPR Explained



But what is CRISPR-Cas9? An animated introduction to Gene Editing. #some2