# **CRISPR** technologies + agriculture + life science applications

COLLEGE of

#### Dr. Rebecca Shapiro **Assistant Professor Department of Molecular and Cellular Biology University of Guelph** OGICAL SCIENCE



#### Hi, I'm **Rebecca** Shapiro



I'm an Assistant Professor in the Department of Molecular and Cellular Biology at the **University of Guelph** 



I studied the genetic determinants of fungal pathogenesis at the **University of Toronto** with **Dr. Leah Cowen**... ...and the development of novel CRISPR-based genetic engineering technologies at **MIT** and **the Broad Institute** with **Dr. Jim Collins** 





Rebecca Shapiro My lab develops CRISPR technologies for genetic manipulation and applications to infectious disease

You can find me shapiror@uoguelph.ca @ShapiroRebecca

### Overview

Introduction to CRISPR (gene editing)

Some historical perspective

Current state of the field

Public perception, fears

Where does Ontario/Canada fit in?





Often described as "molecular scissors" or "scalpel", CRISPR is a genetic engineering technology to precisely edit/alter genes/genomes

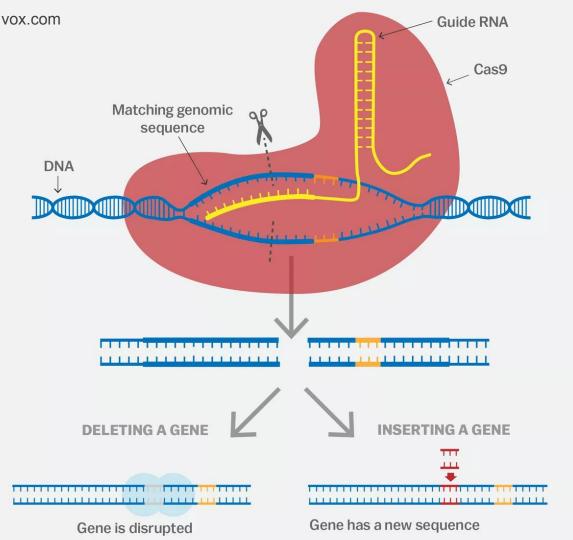
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**CRISPR 101** 

## CRISPR 101

- **Cas9 endonuclease** these are the "molecular scissors" that cut DNA
- **Guide RNA** this short piece of RNA acts as a homing device, targeting Cas9 to precise genomic locations
- **Repair DNA** genetic information that tells the cell how to fix its broken DNA

Facilitates deletion, modification, insertion of desired genetic information



CRISPR 101

vox.com



# CRISPR editing is one of most important biomedical tools developed in recent times

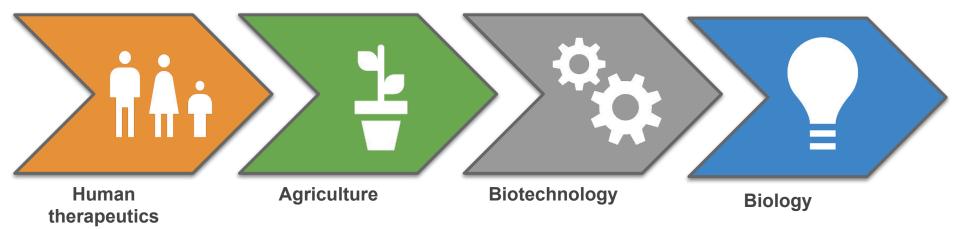
## CRISPR has **revolutionized** genetic biotechnology, precision DNA editing and gene therapy

modification, insertion of desired genetic information

Gene is disrupted

······

Gene has a new sequence



Scientists use CRISPR editing in any living cells (human, plants, animals, insects, microbes...)

CRISPR can be (and is!) used to:

- Repair mutant genes associated with human genetic diseases (i.e. CF, Huntington's)
- Introduce desired new genes into plants (i.e. disease, drought resistance)
- Make malaria-resistant mosquitoes
- Engineer microbes for **biofuel production** and **drug production**

. . .

# So what's the story?

## A brief "history" lesson



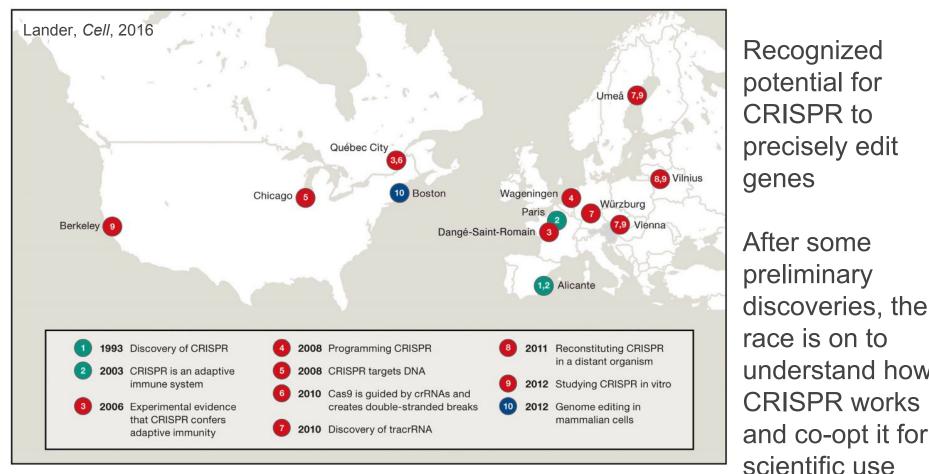
Industrial food scientist are trying to figure out how *Streptococcus thermophilus* bacteria can resist viruses (~2007)

Experimental evolution of virus-sensitive bacteria to select for virus-resistant strains

CRISPR was initially identified as a **naturally-occuring** defence mechanism in bacteria.

χХ

CRISPR (Cas9, guides RNAs) exist in bacteria to target and destroy (break) **foreign invading DNA** (i.e. viruses that attack bacteria)



Recognized potential for **CRISPR** to precisely edit genes After some preliminary discoveries, the race is on to understand how **CRISPR** works

#### Figure 2. The Twenty-Year Story of CRISPR Unfolded across Twelve Cities in Nine Countries

For each "chapter" in the CRISPR "story," the map shows the sites where the primary work occurred and the first submission dates of the papers. Green circles refer to the early discovery of the CRISPR system and its function; red to the genetic, molecular biological, and biochemical characterization; and blue to the final step of biological engineering to enable genome editing.

#### A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,<sup>1,2</sup>\* Krzysztof Chylinski,<sup>3,4</sup>\* Ines Fonfara,<sup>4</sup> Michael Hauer,<sup>2</sup>† Jennifer A. Doudna,<sup>1,2,5,6</sup>‡ Emmanuelle Charpentier<sup>4</sup>‡

17 AUGUST 2012 VOL 337 SCIENCE www.sciencemag.org



In 2012 Jennifer Doudna and Emmanuelle Charpentier show they can co-opt this bacterial CRISPR-Cas9 system for targeted gene editing in the lab (they can target Cas9 to a particular genetic location and cut the DNA)

## CRISPR genome editing in human cells (2013)

#### RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,<sup>1</sup>\* Luhan Yang,<sup>1,3</sup>\* Kevin M. Esvelt,<sup>2</sup> John Aach,<sup>1</sup> Marc Guell,<sup>1</sup> James E. DiCarlo,<sup>4</sup> Julie E. Norville,<sup>1</sup> George M. Church<sup>1,2</sup>†

www.sciencemag.org SCIENCE VOL 339 15 FEBRUARY 2013



Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease

Seung Woo Cho<br/>1–3, Sojung Kim<sup>1–3</sup>, Jong Min ${\rm Kim^{1,2}}$  & Jin-Soo<br/>  ${\rm Kim^{1,2}}$ 



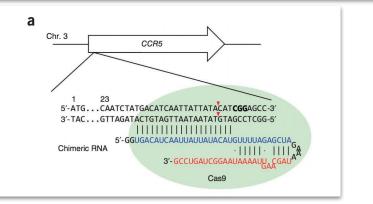
VOLUME 31 NUMBER 3 MARCH 2013 NATURE BIOTECHNOLOGY

#### Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,<sup>1,2</sup>\* F. Ann Ran,<sup>1,4</sup>\* David Cox,<sup>1,3</sup> Shuailiang Lin,<sup>1,5</sup> Robert Barretto,<sup>6</sup> Naomi Habib,<sup>1</sup> Patrick D. Hsu,<sup>1,4</sup> Xuebing Wu,<sup>7</sup> Wenyan Jiang,<sup>8</sup> Luciano A. Marraffini,<sup>8</sup> Feng Zhang<sup>1</sup>†

Sciencexpress / http://www.sciencemag.org/content/early/recent / 03 January 2013 / Page 1/ 10.1126/science.1231143





Transformed **human cell lines** Cas9, guide RNAs targeting genes of interest, and repair DNA. Example, mutated *CCR5* gene involved in resistance to HIV

Current state of affairs

Academic labs are working towards new CRISPR applications

Many new companies have formed on CRISPR therapeutics and other applications

Patent battles affecting utilization of this technology

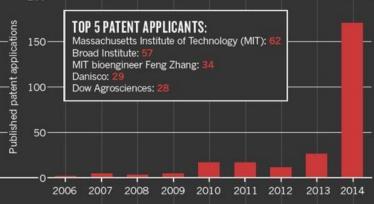
# Current state of affairs



#### PATENTS

-200

In 2014, worldwide patent applications that mention CRISPR leapt and a patent battle intensified.



#### FUNDING

A sharp jump in US National Institutes of Health funding for projects involving CRISPR is a harbinger of future advances.



Academic labs are working towards new CRISPR applications

Many new companies have formed on CRISPR therapeutics and other applications

#### Patent battles affecting utilization of this technology

#### State of CRISPR commercial activities



Three biggest companies founded by academics from early CRISPR discoveries Now countless other companies and startups focusing on health, agriculture applications

#### Patent wars over CRISPR-Cas9 technology



One the most heated disputes between two educational institutions over CRISPR-Cas9 patent

In September 2018 US Court of Appeals for the Federal Circuit awarded the intellectual property to the Broad Institute for **CRISPR-Cas9 genome editing in eukaryotic cells** (including plant, animal, and human)

In February 2019, Berkley is granted a CRISPR patent for use in any cellular or non-cellular environment

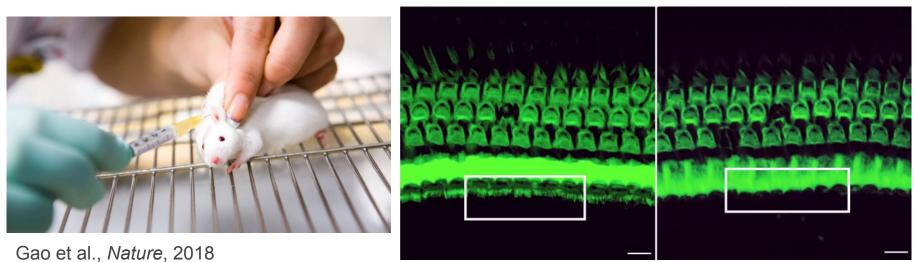
Door left open for other CRISPR-based editing technologies (i.e. other non-Cas9 enzymes)



# **CRISPR + applications to human health**



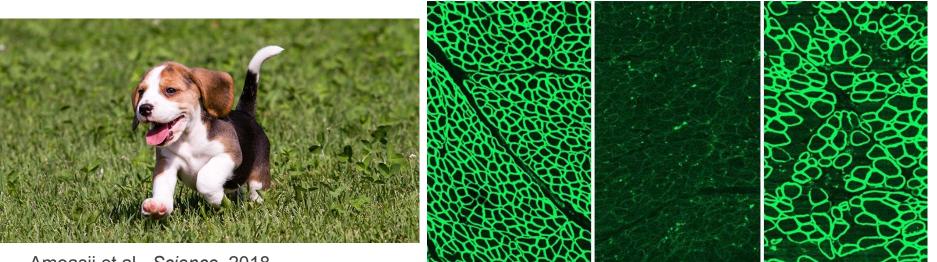
### Curing disease with CRISPR engineering



CRISPR targeting mutant *TMC1* gene (involved in deafness) applied in mice ears, restored ear cell hairs and hearing

Cas9 protein and guide RNAs encased in lipid delivery system

### Curing disease with CRISPR engineering



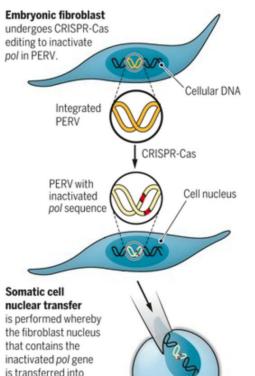
Amoasii et al., Science, 2018

CRISPR targeting mutant *DMD* gene injected in to young dogs helps cure symptoms of Duchenne muscular dystrophy

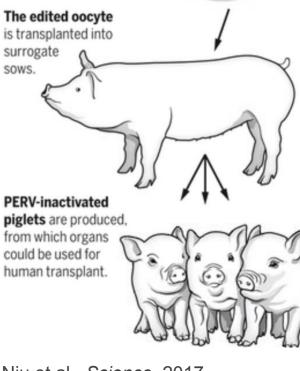
Cas9 protein and guide RNAs delivered with (harmless) adeno-associated viruses (Similar technique showed promising results in mice ALS studies)

## **CRISPR** applications beyond humans

Producing PERV-inactivated pigs



a denucleated oocyte.



CRISPR targeting Porcine Endogenous Retroviruses (PERVs) inactivates all the PERV viruses simultaneously

PERV-free pig organs could be used for transplants in humans

Niu et al., Science, 2017

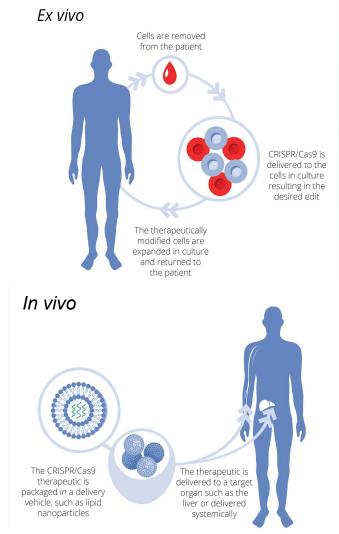
	Program	Editing approach	Research	IND-enabling	Ph I/II	Partner	Structure
	Ex vivo: Hematopoietic						
	CTX001: β-thalassemia	Disruption			CT. Appro		Collaboration
CRISPRI	CTX001: Sickle cell disease (SCD)	Disruption		$\rangle \rightarrow$	CT Appro		Collaboration
THERAPEUTICS	Hurler syndrome (MPS-1)	Correction					Wholly-owned
THERAPEOTICS	Severe combined immunodeficiency (SCID	) Correction					Joint venture
	Ex vivo: Immuno-oncology						
VERTEX	CTX110: Anti-CD19 allogeneic CAR-T	Various			IND f		Wholly-owned
	CTX120: Anti-BCMA allogeneic CAR-T	Various					Wholly-owned
	CTX130: Anti-CD70 allogeneic CAR-T	Various					Wholly-owned
	Ex vivo: Regenerative Medicine						
	Type I diabetes mellitus	Various				VIACYTE	Collaboration
	In vivo: Liver						
	Glycogen storage disease Ia (GSD Ia)	Correction					Wholly-owned
	Hemophilia	Correction				CABEBIA	Joint venture
	In vivo: Other organs						
	Duchenne muscular dystrophy (DMD)	Disruption					Wholly-owned
	Cystic fibrosis (CF)	Correction				VERTEX	License option

In September 2018 CRISPR Therapeutics and Vertex launched the first US/European clinical trials for CRISPR (**CTX001** targeting Beta-Thalassemia/Sickle Cell Disease)

Many more in the pipeline for CRISPR Therapeutics, Editas, etc. Targeting CF, DMD, hereditary blindness, others.

Other CRISPR-based cancer clinical trials are ongoing in China

All these trials are in adults with disease



## **CRISPR-edited** twins

November 2018 He Jiankui <sup>ge</sup> announced twin girls from **CRISPR-edited embryos** were born in China

Babies have mutants in *CCR5* gene involved in resistance to HIV

Unlike adult gene editing, gene will be mutated in all cells, with unknown side effects



# **CRISPR +** applications to agriculture



### Non-browning crops

In 2016 the white button mushroom becomes the first CRISPR-edited organism to be approved by the USDA

Has an enzyme (polyphenol oxidase, PPO) knocked out making it resistant to browning



#### 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

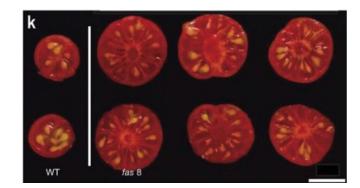
14 April 2016



#### **CRISPR** tomatoes

2018: Wild tomato species 'domesticated' with CRISPR mutations

Altered tomato size, and yield while maintaining desired properties of wild species (flavours, nutritional value)





#### **CRISPR** waxy corn

DowDuPont knocking out amylose enzyme in corn crops with CRISPR

Boosts kernel starch content >97% amylopectin compared with 75% in regular corn - waxy corn trait in elite corn strains without compromising yield



## **CRISPR** applications in agriculture



CRISPR editing to boost the **cacao** plant's immune system to viruses

CRISPR-generated **bananas** that are resilient to a deadly fungal pathogen

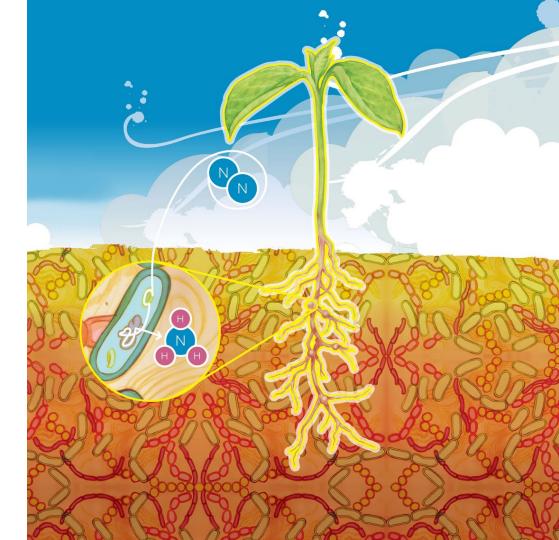
CRISPR-made **wheat** crops that are lower in gluten proteins

. .

# CRISPR editing in microbes for agriculture

Bayer/Ginkgo Bioworks joint venture Joyn aims to reduce costly use of nitrogen fertilizer

Using gene editing to engineer microbes ability to fix Nitrogen for cereal crops



### **CRISPR editing in animals**

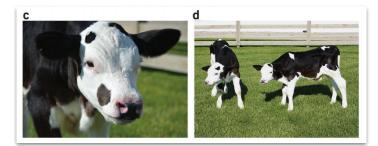
Recombinetics: CRISPR editing cows that are born without horns, avoiding need for dehorning procedures

#### nature biotechnology

Correspondence | Published: 06 May 2016

#### Production of hornless dairy cattle from genome-edited cell lines

Daniel F Carlson, Cheryl A Lancto, Bin Zang, Eui-Soo Kim, Mark Walton, David Oldeschulte, Christopher Seabury, Tad S Sonstegard & Scott C Fahrenkrug 🔀





#### 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

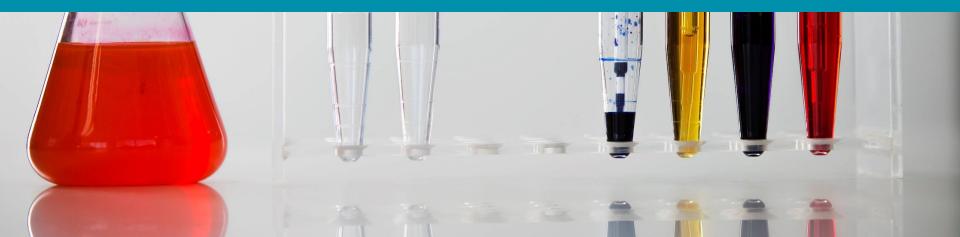


Credit : iStock/Rasica

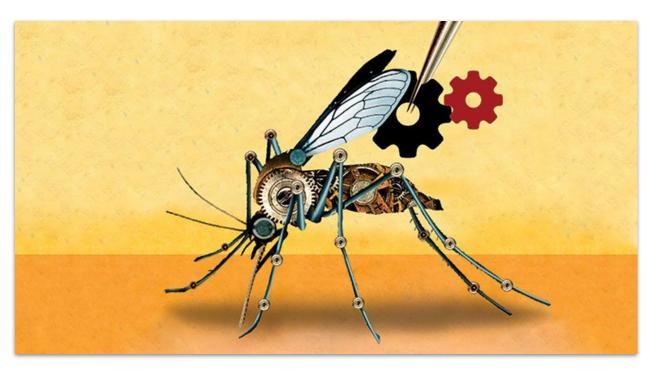




# **CRISPR + other applications**

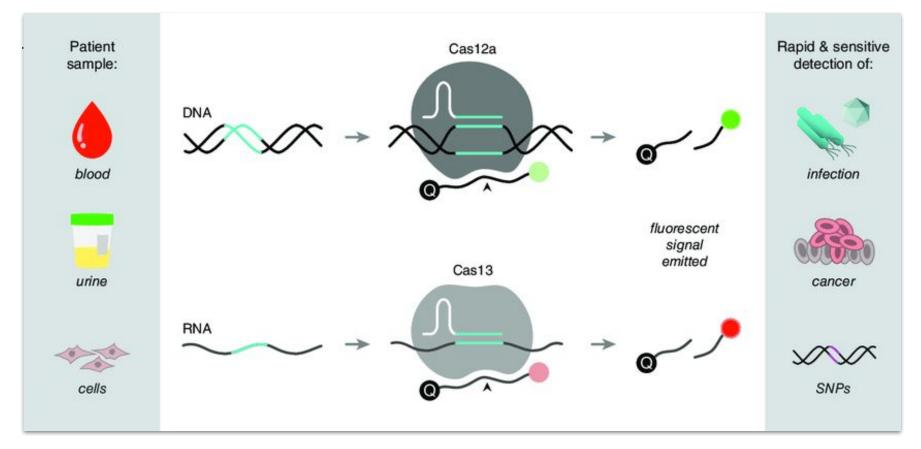


### **CRISPR** applications beyond humans and plants



CRISPR editing in mosquitoes to prevent malaria parasite carriage

### **CRISPR-based diagnostics**



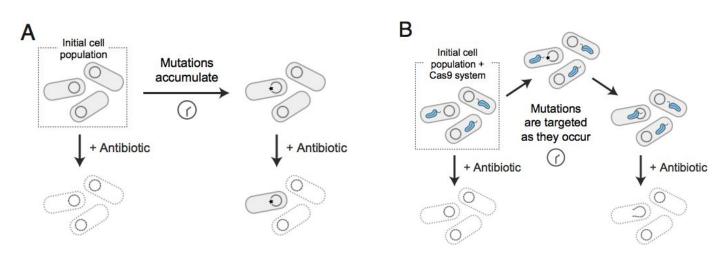
#### **CRISPR-based biofuels**



Synthetic Genomics/ExxonMobil generated a CRISPR engineered algal strain with double lipid content

#### Towards commercialization of algae-based biofuels

### **CRISPR-based antimicrobials**



CRISPR-based antimicrobials: using CRISPR to targetedly delete virulence, antibiotic resistance factors from infectious pathogens

# Controversies and public perception

Gene editing always brings up public fears surrounding modifying genetic information:

- Food GMOs
- Germline editing, "designer babies"
- General health and safety
- DIY CRISPR



## **Food-related GMOs**

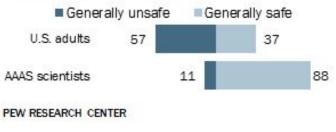
Many important food (plant and animal) CRISPR projects underway

A majority (of Americans) think GMOs are unsafe

In Canada, concerns about GMO labelling, though non-CRISPR GMO products now coming to market

### Eating Genetically Modified Foods

% of each group saying it is generally safe or unsafe to eat genetically modified foods



The Strategic Counsel

No strong rationale for GM foods has been presented to the public which exacerbates existing questions and concerns.



labeling is not viewed as credible)



## **Regulation of CRISPR crops**



Crop researchers worry a new European court ruling could complicate efforts to move gene-edited crops from the lab to the field. MICHAEL GOTTSCHALK/PHOTOTHEK/GETTY IMAGES

### European court ruling raises hurdles for CRISPR crops

By Erik Stokstad | Jul. 25, 2018 , 4:40 PM



U.S. DEPARTMENT OF AGRICULTURE

### WTO Members Support Policy Approaches to Enable Innovation in Agriculture



In Japan, genetically modified products have to be labeled; an advisory panel did not say whether that should apply to gene-edited food as well. SHIHO FUKADA/BLOOMBERG/GETTY IMAGES

Gene-edited foods are safe, Japanese panel concludes

By Dennis Normile | Mar. 19, 2019 , 1:15 PM

Science Home News Journals To

#### BIOLOGY

### USDA confirms it won't regulate CRISPR gene-edited plants like it does GMOs

Rich Haridy | April 3rd, 2018



A recent USDA statement has clarified that the agency views CRISPR gene-edited plants as very different to genetically modified organisms and suggests no regulation need be imposed on the industry (Credit: Matic.Sandra/Depositphotos)

Different rulings on CRISPR crops - implications for international trade

## Germline editing

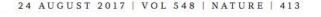
- Using CRISPR editing for germline cells (egg, sperm) or embryos before implantation
- Consequences for disease traits and other heritable traits
- China moving fastest in germline/embryo CRISPR editing
- Last year US group announced foundational work editing a gene involved in heart disease in human embryos with CRISPR

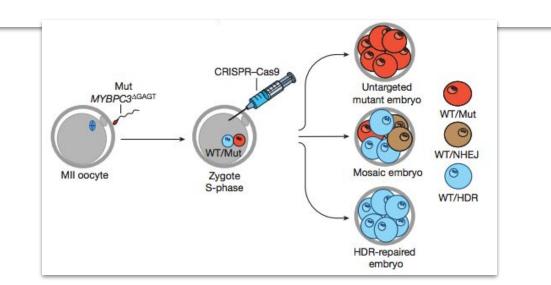
### ARTICLE

doi:10.1038/nature23305

### Correction of a pathogenic gene mutation in human embryos

Hong Ma<sup>1</sup>\*, Nuria Marti-Gutierrez<sup>1</sup>\*, Sang-Wook Park<sup>2</sup>\*, Jun Wu<sup>3</sup>\*, Yeonmi Lee<sup>1</sup>, Keiichiro Suzuki<sup>3</sup>, Amy Koski<sup>1</sup>, Dongmei Ji<sup>1</sup>, Tomonari Hayama<sup>1</sup>, Riffat Ahmed<sup>1</sup>, Hayley Darby<sup>1</sup>, Crystal Van Dyken<sup>1</sup>, Ying Li<sup>1</sup>, Eunju Kang<sup>1</sup>, A.-Reum Park<sup>2</sup>, Daesik Kim<sup>4</sup>, Sang-Tae Kim<sup>2</sup>, Jianhui Gong<sup>5,6,7,8</sup>, Ying Gu<sup>5,6,7</sup>, Xun Xu<sup>5,6,7</sup>, David Battaglia<sup>1,9</sup>, Sacha A. Krieg<sup>9</sup>, David M. Lee<sup>9</sup>, Diana H. Wu<sup>9</sup>, Don P. Wolf<sup>1</sup>, Stephen B. Heitner<sup>10</sup>, Juan Carlos Izpisua Belmonte<sup>3</sup>§, Paula Amato<sup>1,9</sup>§, Jin-Soo Kim<sup>2,4</sup>§, Sanjiv Kaul<sup>10</sup>§ & Shoukhrat Mitalipov<sup>1,10</sup>§





### Call for a global moratorium on CRISPR germline gene editing



Embryos cultured as part of in vitro fertilization can be screened for genetic diseases.

# Adopt a moratorium on heritable genome editing

Eric Lander, Françoise Baylis, Feng Zhang, Emmanuelle Charpentier, Paul Berg and specialists from seven countries call for an international governance framework.

## Safety concerns

- Recent concerns regarding how CRISPR editing might introduce unwanted errors, mutations, or deletions into the human genome
- With continual progress towards more targeted, specific, accurate technologies, problems are likely surmountable
- Newer techniques (base editing, repression) may prove less error-prone

### BRIEF COMMUNICATIONS ARISING

### Large deletions induced by Cas9 cleavage

ARISING FROM H. Ma et al. Nature 548, 413-419 (2017); https://doi.org/10.1038/nature23305

E8 | NATURE | VOL 560 | 9 AUGUST 2018

LETTERS

#### nature biotechnology

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

Michael Kosicki, Kärt Tomberg & Allan Bradley

NATURE BIOTECHNOLOGY ADVANCE ONLINE PUBLICATION

# What's happening in Canada

Recent push to emphasize 'Synthetic Biology' (i.e. genetic engineering with CRISPR, etc)

- New programs, community networks, meetings
- New student initiatives



### 7 Ontario iGEM teams (16 Canada)

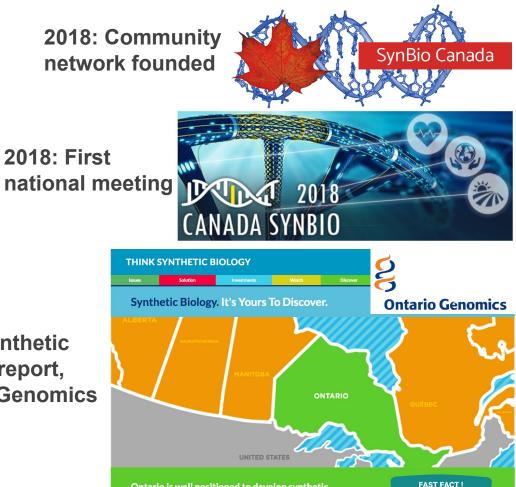
Concordia





New synbio training programs (Western, Concordia)

**2016: Synthetic Biology report**, **Ontario Genomics** 



The Synthetic Biology Leadershi Council, co-chaired by their Min

biology in the U.K.

of Life Sciences, has spearheaded he rapid growth of syntheti

Ontario is well positioned to develop synthetic biology solutions. We have a world-class understanding of biology in place and strong industry sectors that can lend support.

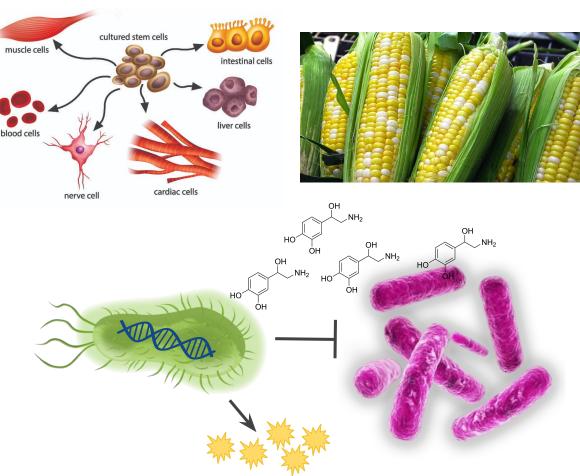
## What's happening in Canada

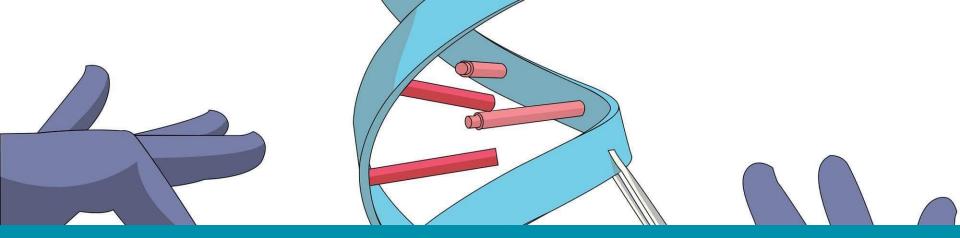
Complementary to existing strengths in life science research areas in Ontario (stem cell research, agriculture, genetic diseases)

Germline CRISPR editing laws in Canada amongst the most restrictive in the world, criminal offence

CFIA regulations favourable to engineered crops - based on trait and not process

Focus on microbes as a target for editing (i.e. CRISPR 'probiotics' to diagnose or treat disease)





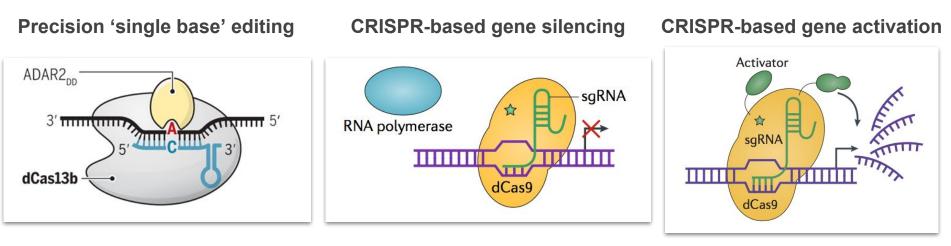
# Questions?



Delivery of CRISPR?

\_ \_ \_

## **Recent advances in CRISPR technology**



Yang & Chen, Science, 2017

Shapiro et al, Nature Reviews Microbiology, 2018

...and countless other technologies currently being developed