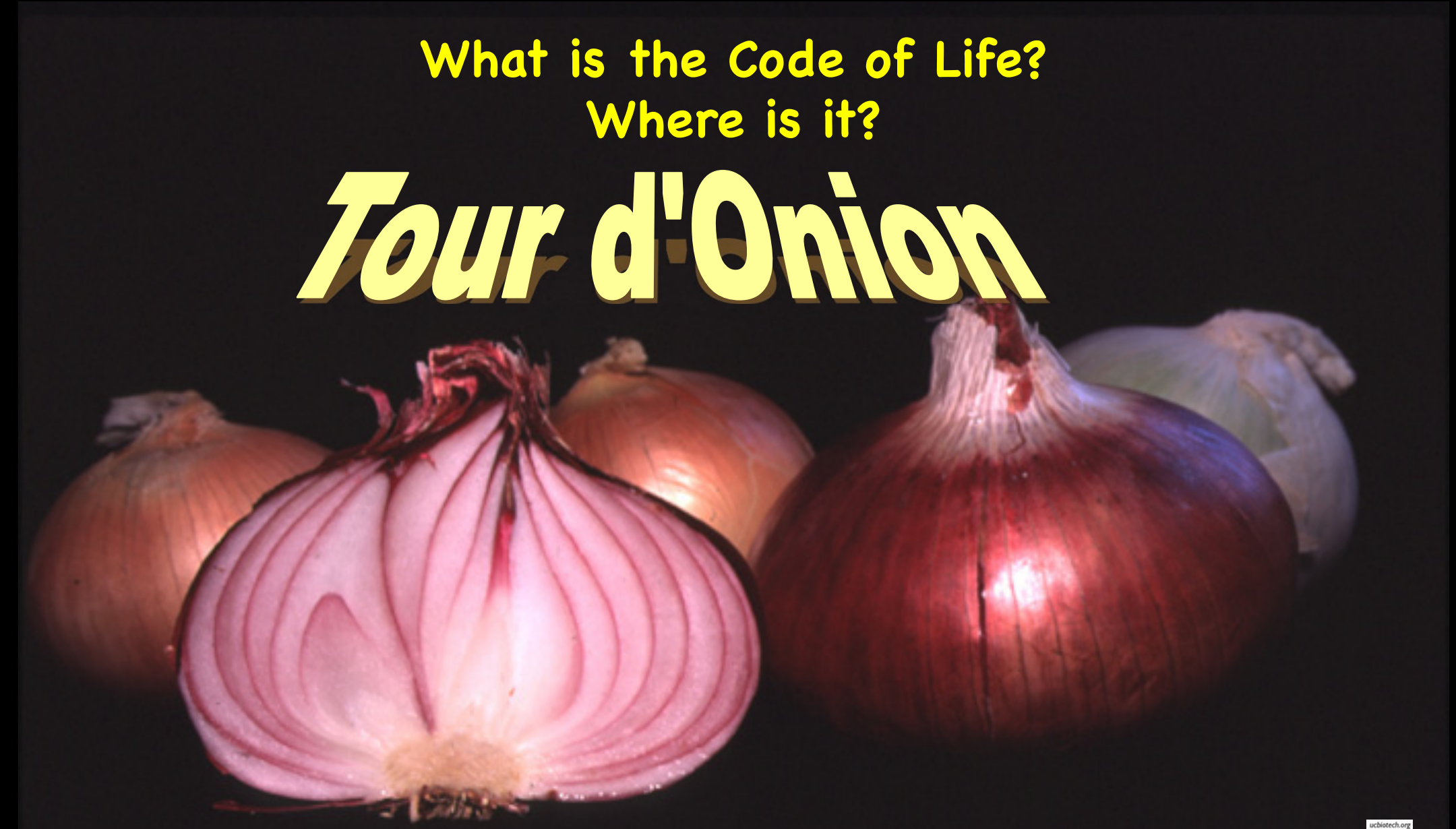
Genetic Modification – Ag Applications in the Code of Life

How many of you played the 1980's Rampage arcade game where gigantic monsters try to survive the onslaught of military forces? Guess what? We have a modern incarnation of that game in a movie. But this time we are told how the monsters get so big!

Peggy G. Lemaux, Ph.D.
University of CA, Berkeley
http:///ucbiotech.org
http://pmb.Berkeley.edu/lemaux

What is the Code of Life?
Where is it?

Tour d'Onion



Or what makes an onion, an onion?

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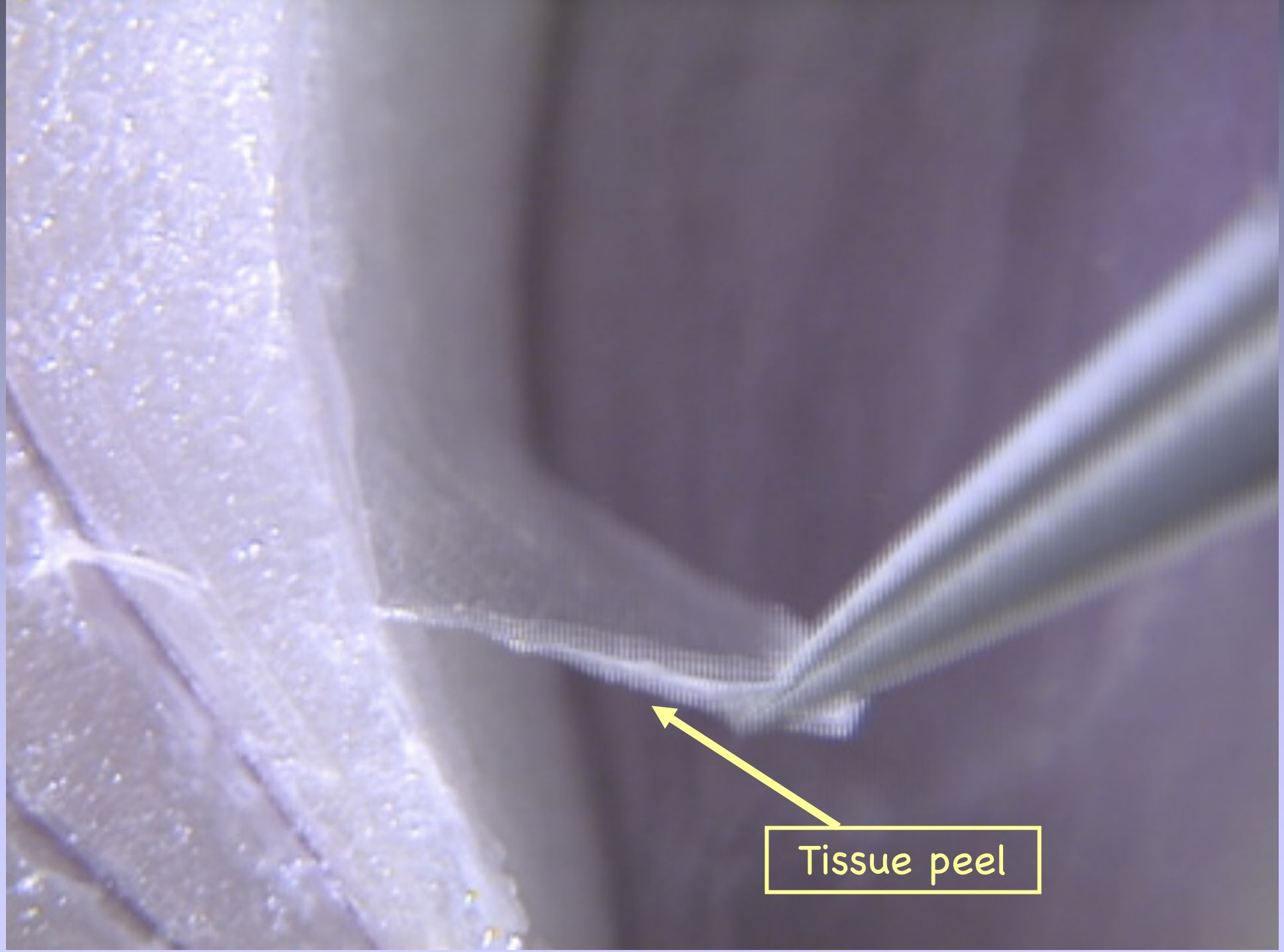
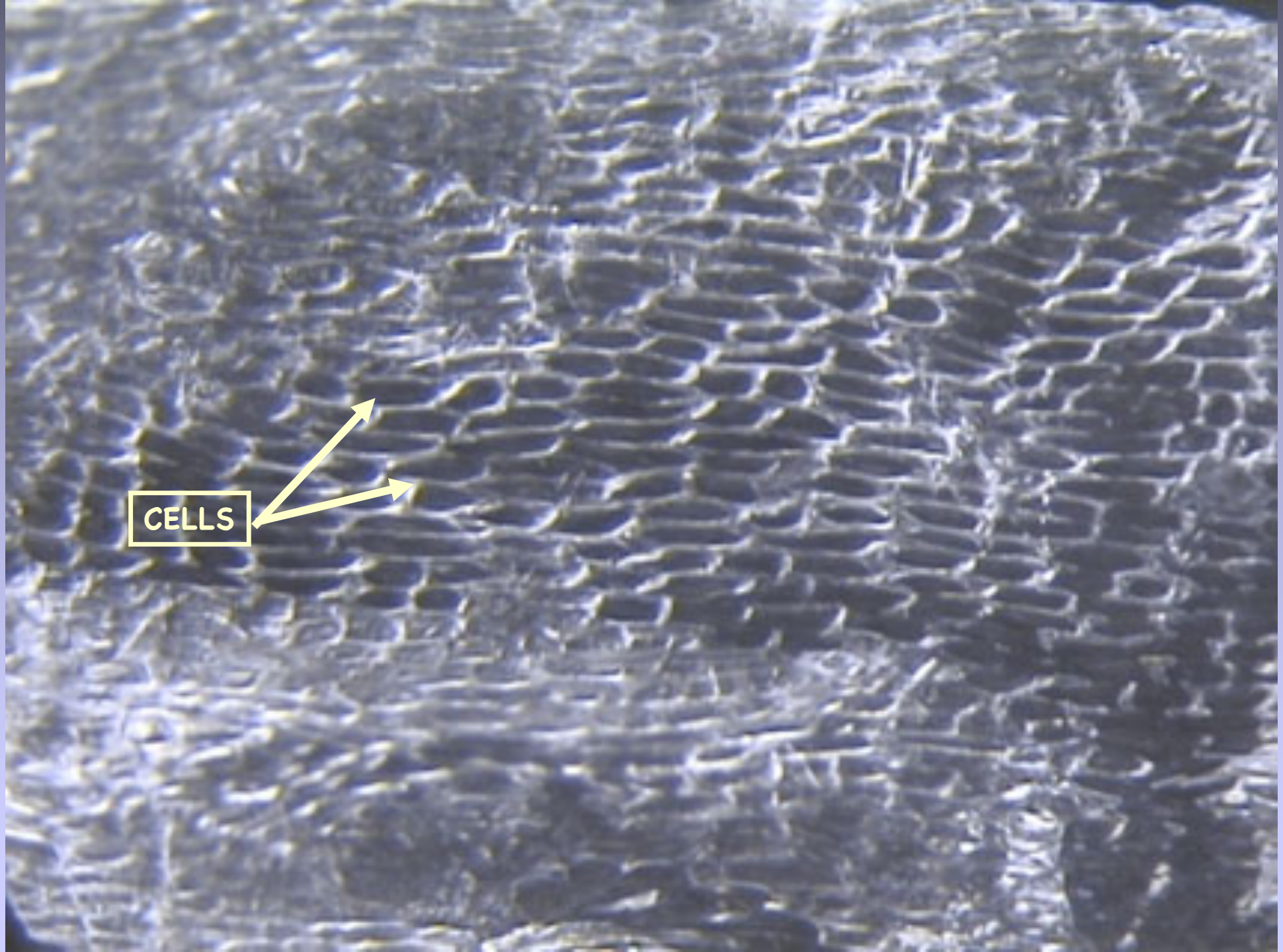
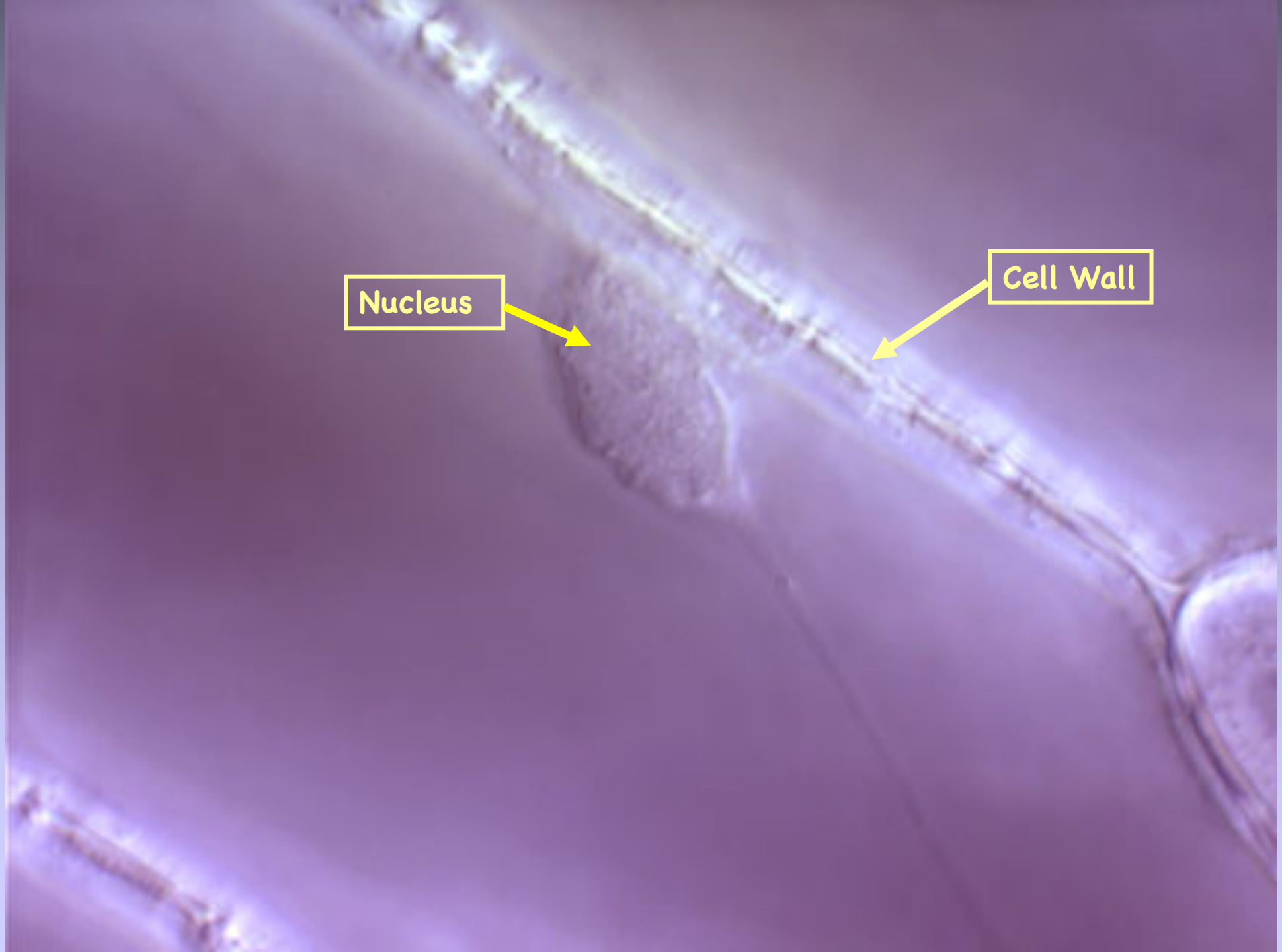
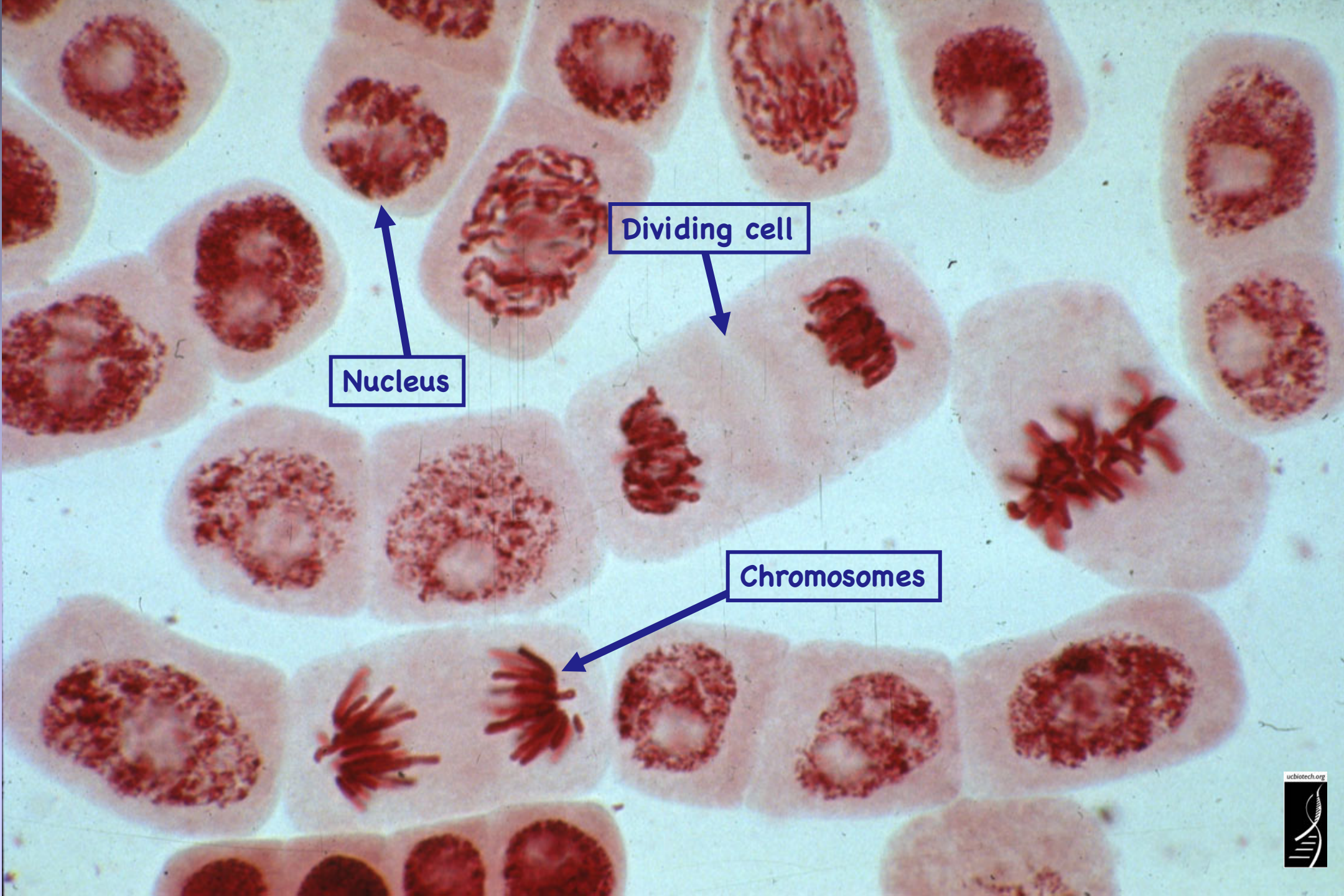
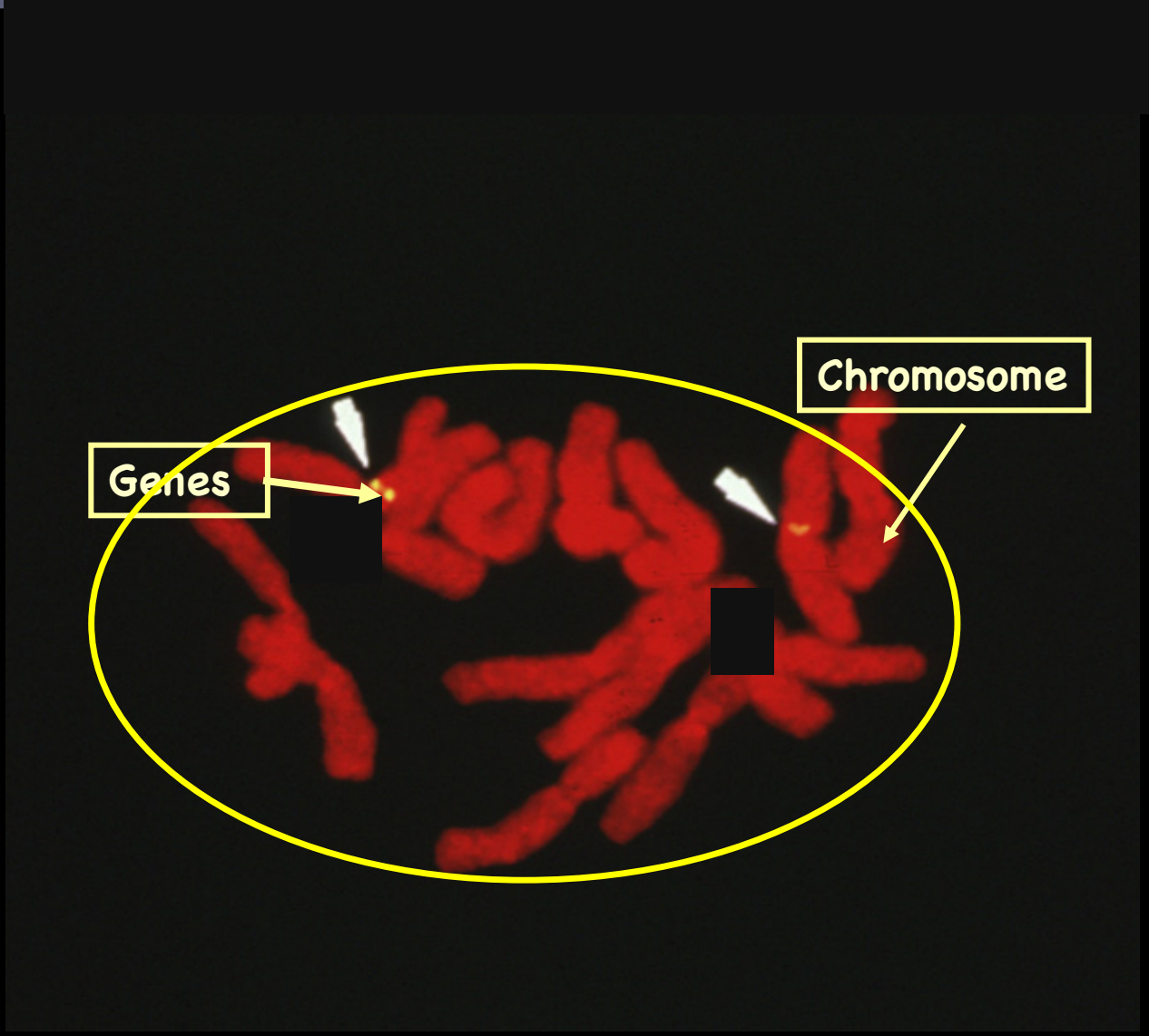

Tissue peel


CELLS


Nucleus
Cell Wall


Dividing cell
Nucleus
Chromosomes



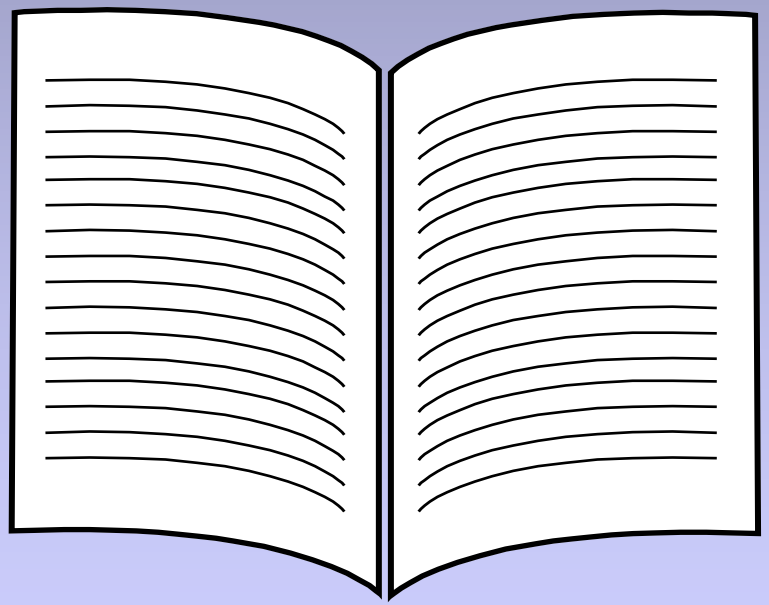
All Chromosomes = Genome = Code of Life

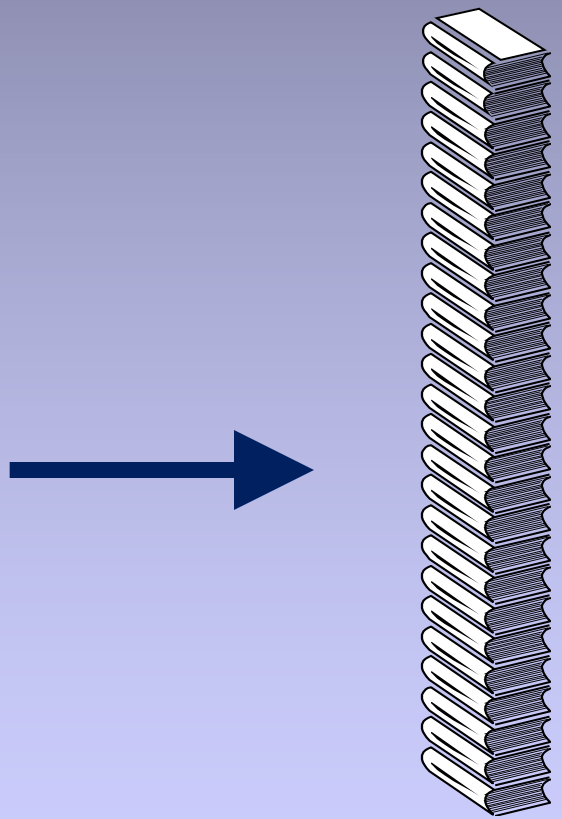
Genetic information in the genome is the "code of life", dictating traits

Let's represent chemical units in that information by alphabetic letters

...CTGA**C**CTAATGCC**G**TA...

...CTGA**A**CTAATGCC**T**TA...





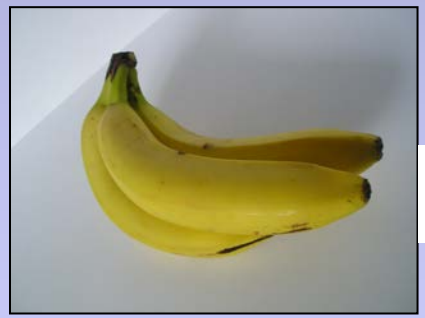
Sometimes mistakes happen when copying information creating changes, called mutations

Mutations Have Gotten These Plants from Looking Like This...

To Looking Like They Are Now

Carrot

Eggplant



Banana

Broccoli,
Kale,
Cabbage

Since 1950's, intentional mutation breeding has created >3200 officially released, genetically modified crops – like 600 maize, rice, wheat varieties.

Photo by Stephen Ausmus, USDA

But genes and chromosomes have also been changed to create new plant varieties by classical breeding?

Triticum monococcum

Ancient variety

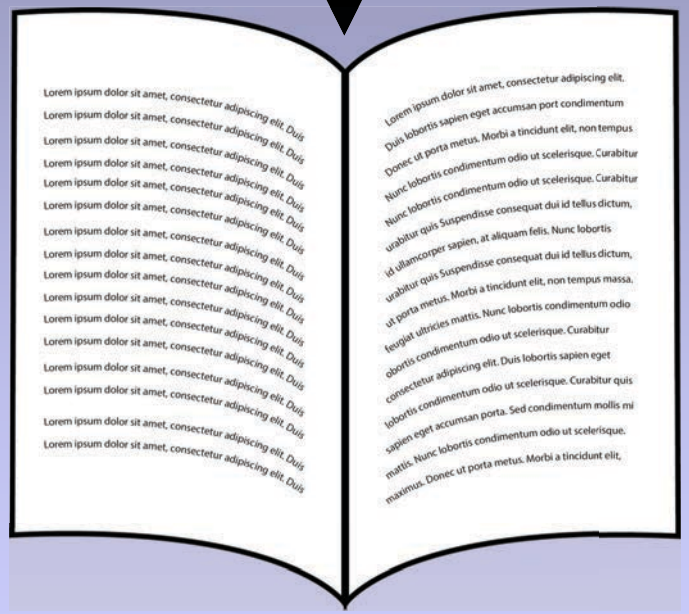
Triticum aestivum

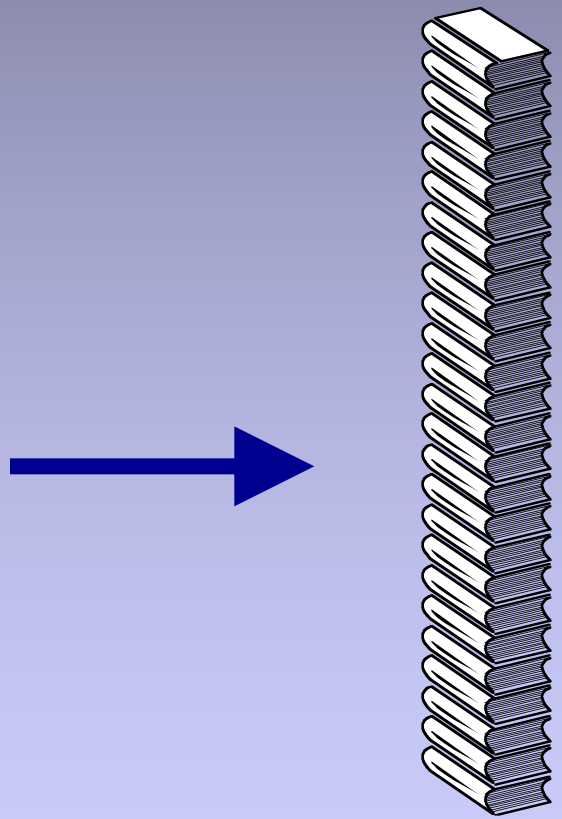
Modern bread variety

Information in the wheat genome

Chemical units represented by alphabetic letters

...CTGACCTAATGCCGTA...





1700 books
1000 pages each

1700 books
(or 1.7 million pages)

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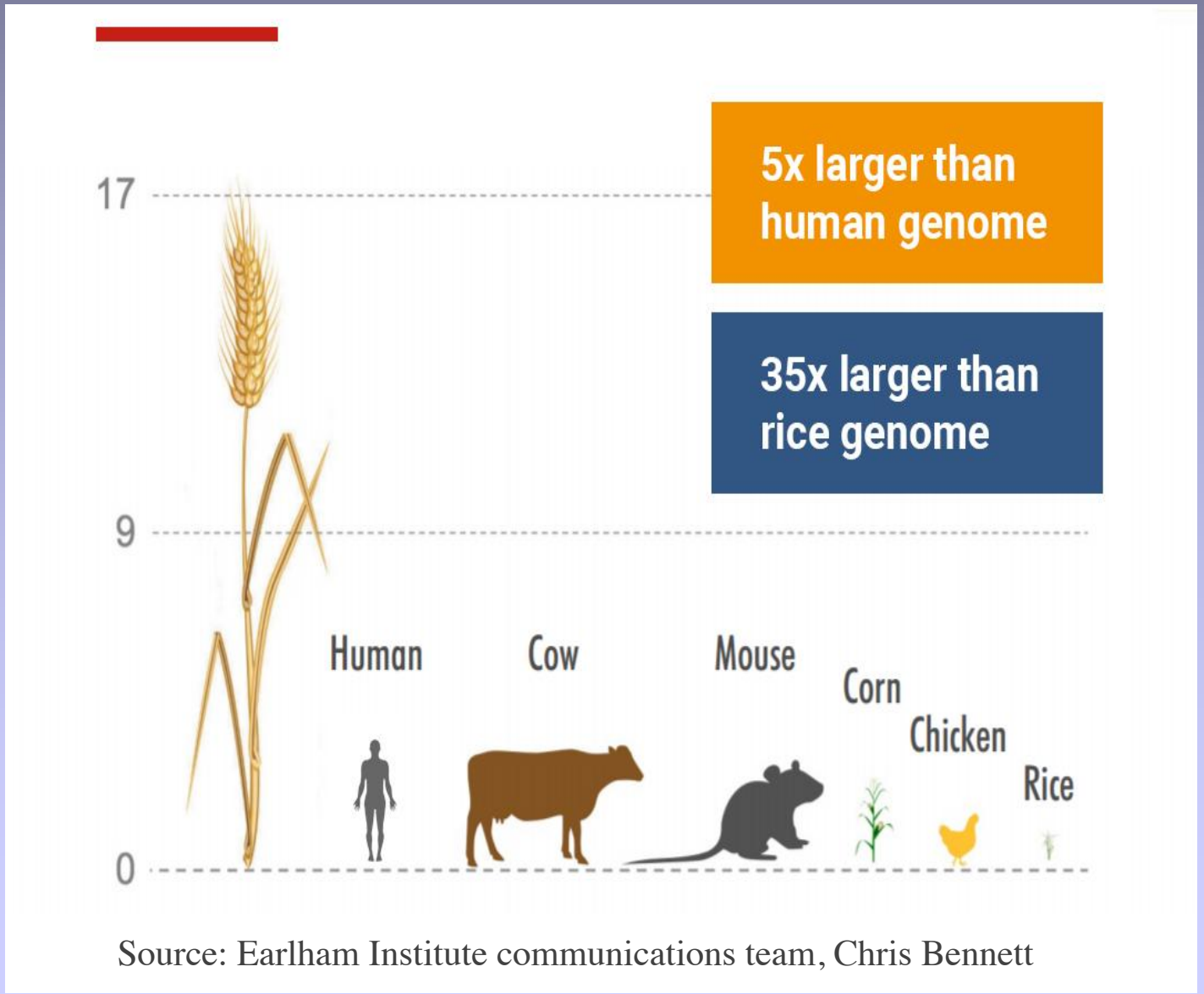
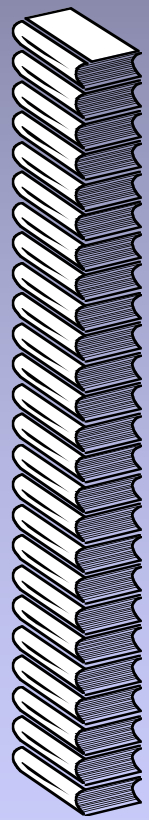
How do genomes compare? Wheat's genome is bigger than human's?

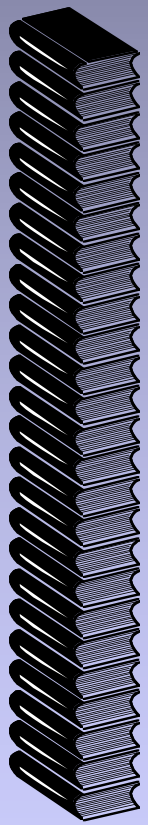


Source: Earlham Institute communications team, Chris Bennett

Hybridization or cross breeding of wheat



X



Random retention of information from each parent

1700 books
(or 1.7 million pages)

1700 books
(or 1.7 million pages)

1700 books
(or 1.7 million pages)

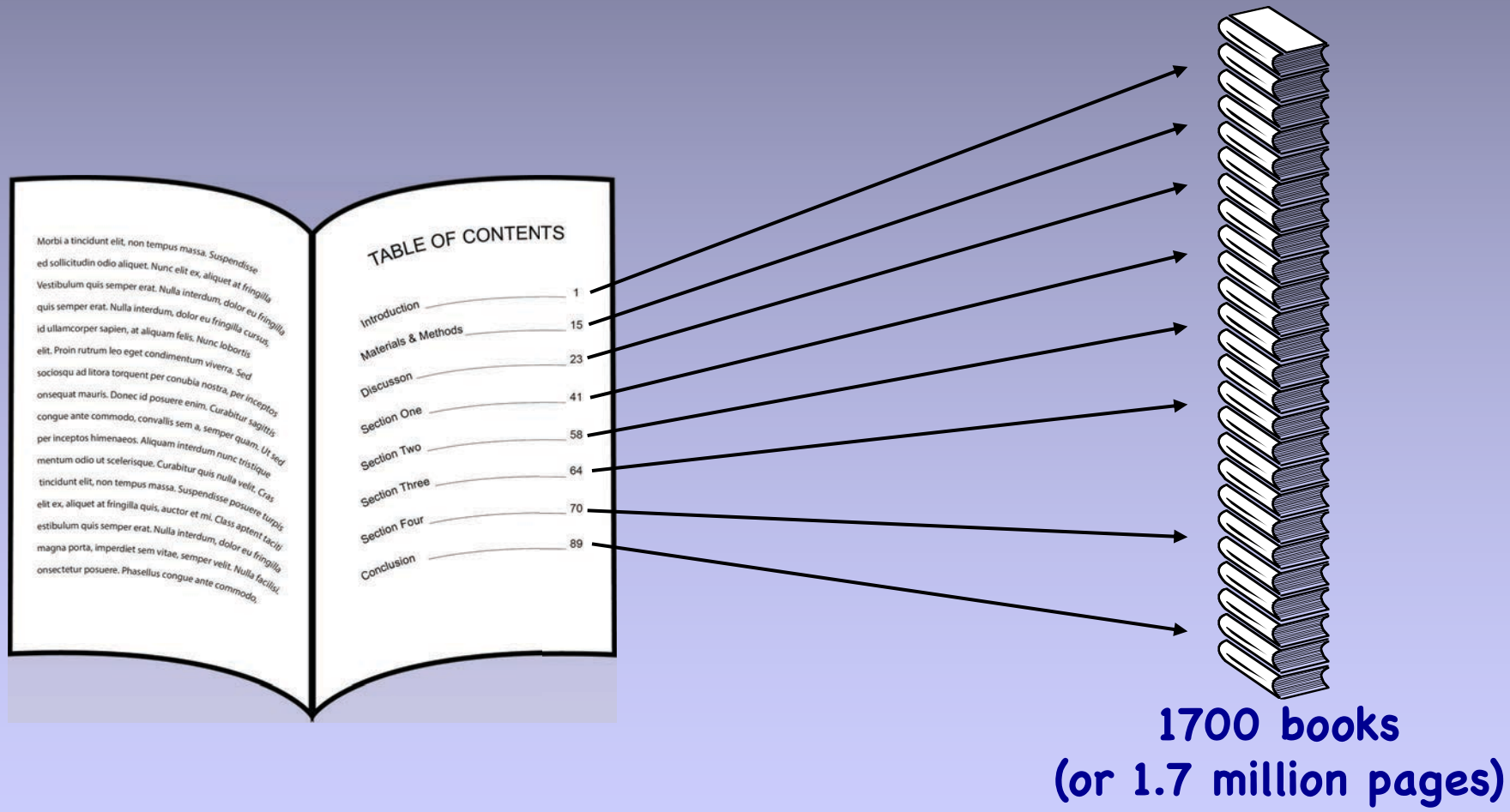
Genetic modification by hybridization <u>is not</u> GE_n or GMO

Putting this in context, these breeding efforts were critical to increasing crop production…

Product	2014 acreage	Acreage needed at 1950's rate	Additional Resources needed
Soybeans 	82,591,000 acres	180,971,889 acres	~98 M acres (= size CA)
Corn 	83,136,000 acres	372,134,346 acres	~289 M acres (= 3X size CA)
Broiler Chickens 	8,544,100,000 head	16,679,545,455 head	~8 B chickens requiring 81.5 B lbs feed

New breeding methods

Use table of contents of genes for marker assisted selection



Genetic modification that is not GE or GMO

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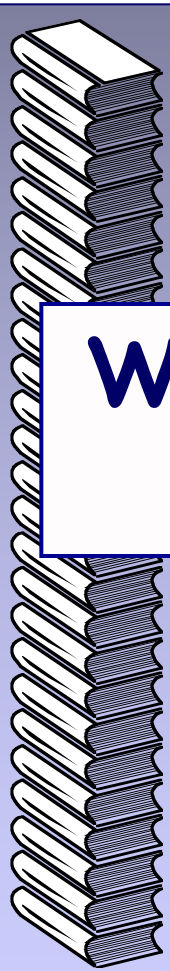
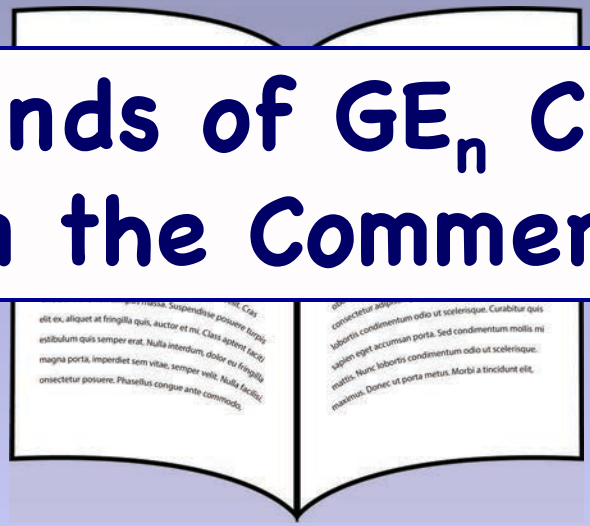
Can't we just do all modifications this way?

Marker-assisted selection used to protect rice against bacterial blight and blast disease

Protection limited to diversity in crop and compatible relatives

Another way to modify genomes uses genetic engineering to create "GMOs"





Inserts randomly in genome

Inserted gene(s)

What Kinds of GE_n Crops and Foods Are in the Commercial Market?

One-half page equivalent to a gene

1700 books (or 1.7 million pages)

Genetic modification that is GE_n or GMO

1700 books (or 1.7 million pages)

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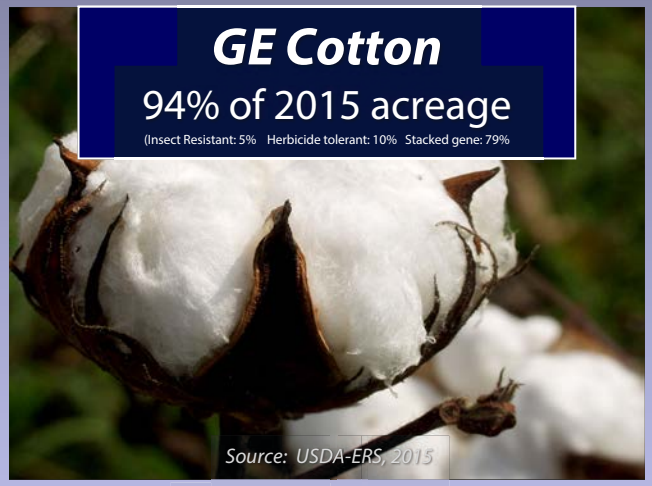
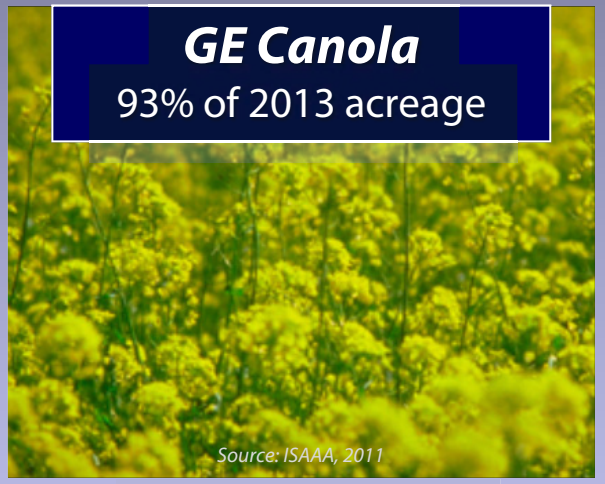
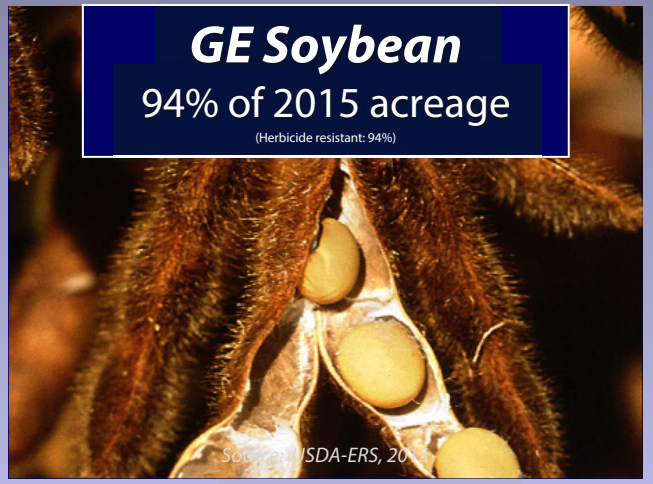
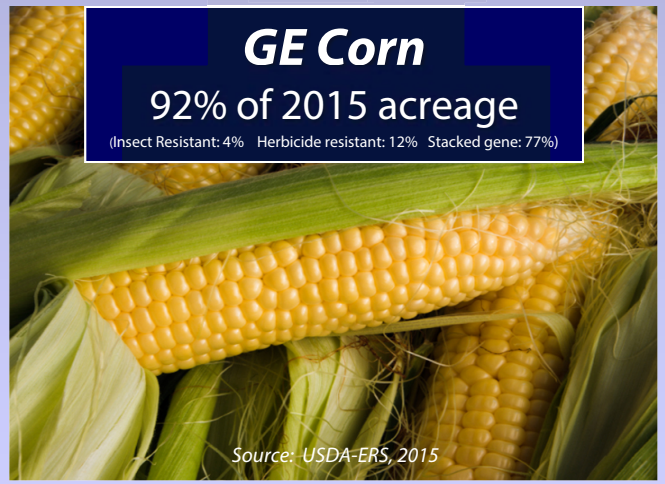
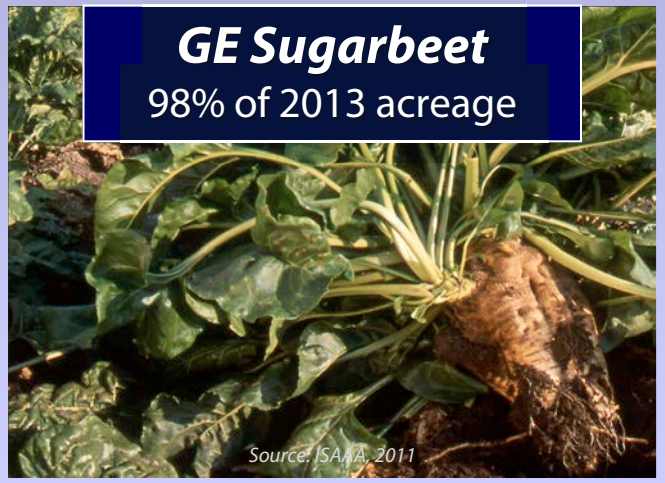
Number of different commercially available, large acreage GE_n (GMO) crops is limited

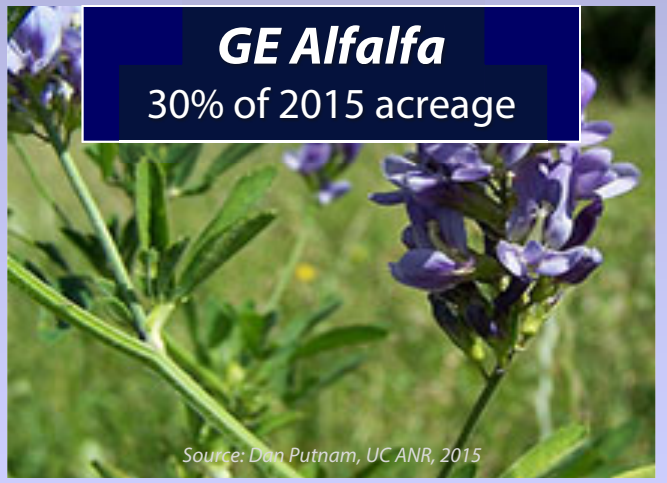












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Number of different traits available in large acreage GE_n crops is also limited

Insect-tolerant Bt crops - engineered for resistance using gene from naturally occurring bacterium

Herbicide-tolerant - engineered with gene to tolerate herbicide application

Crops with stacked traits - both Bt and HT - are available

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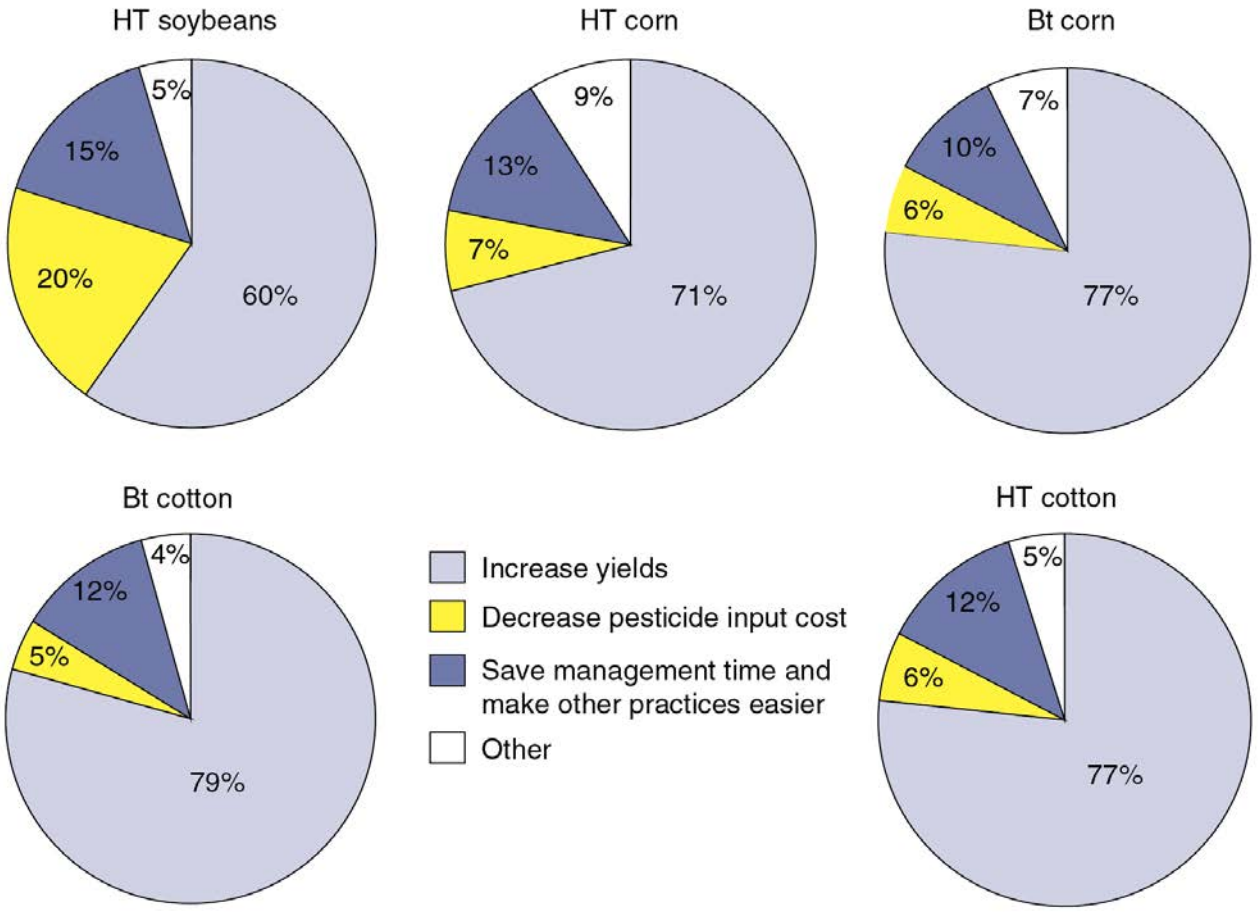
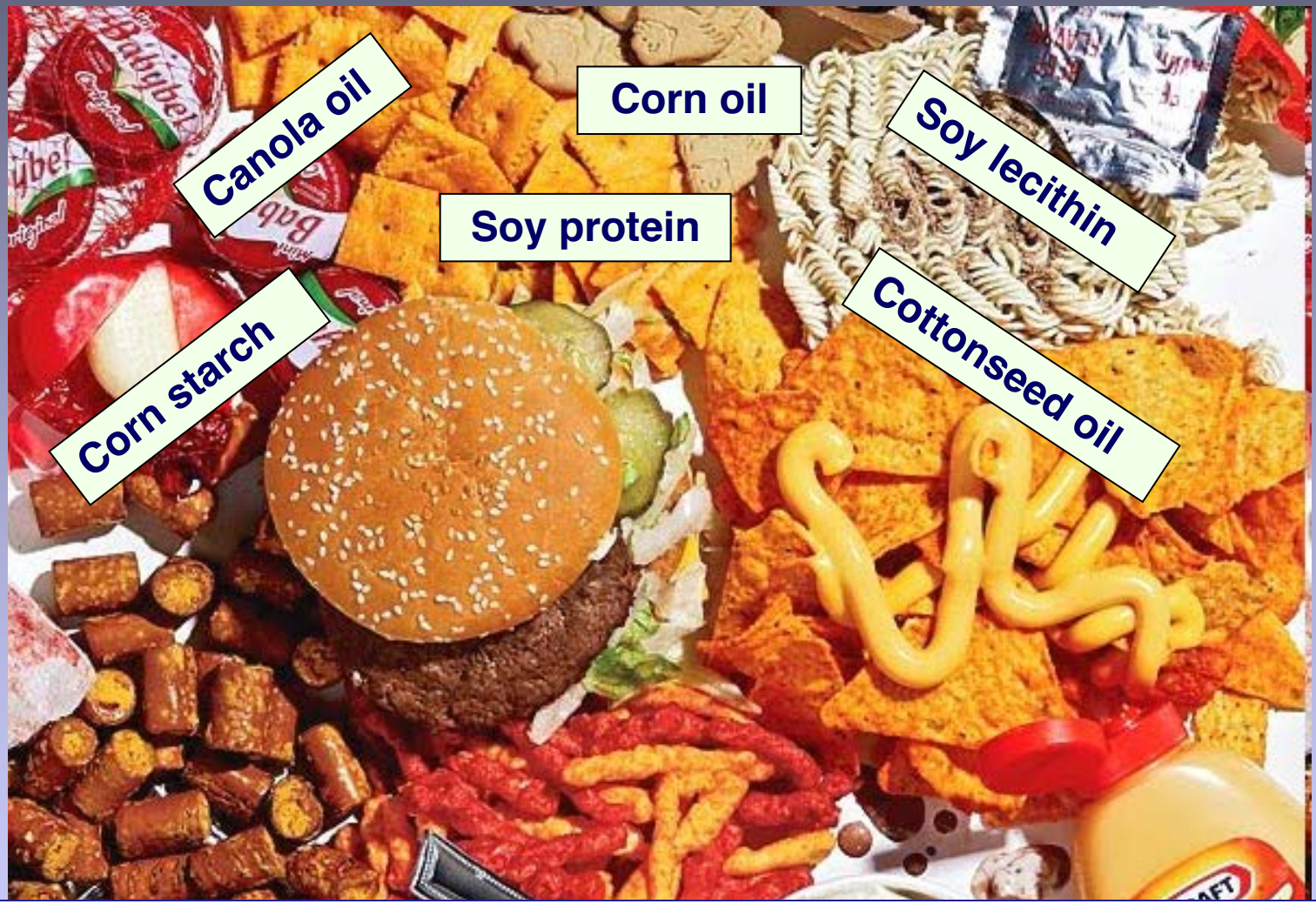
Why do U.S. growers use GE_n crops?



Reasons vary from crop-to-crop but primary reason is improved yields

SOURCE: Fernandez-Cornejo, J., Wechsler, S., Livingston, M. and Mitchell, L. 2014. Genetically Engineered Crops in the United States. USDA Economic Research Service Report No. 162, February 2014.

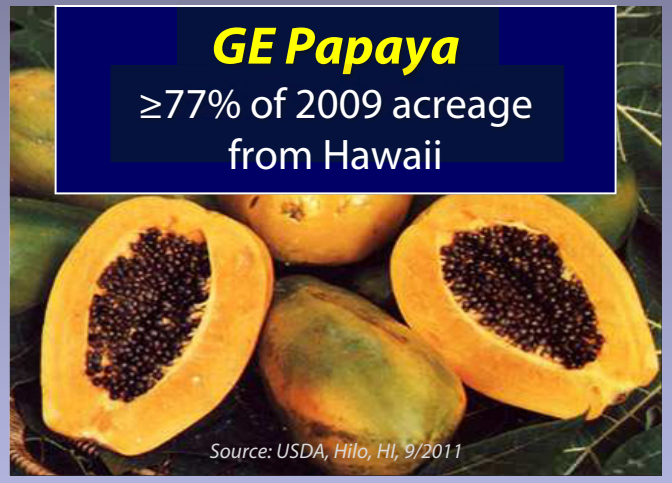


These types of large-acreage GE_n crops lead to estimates that 60-80% of processed foods in U.S. have GE_n ingredients – often only a minor ingredient

SOURCE: https://factsaboutgmos.org/disclosure-statement

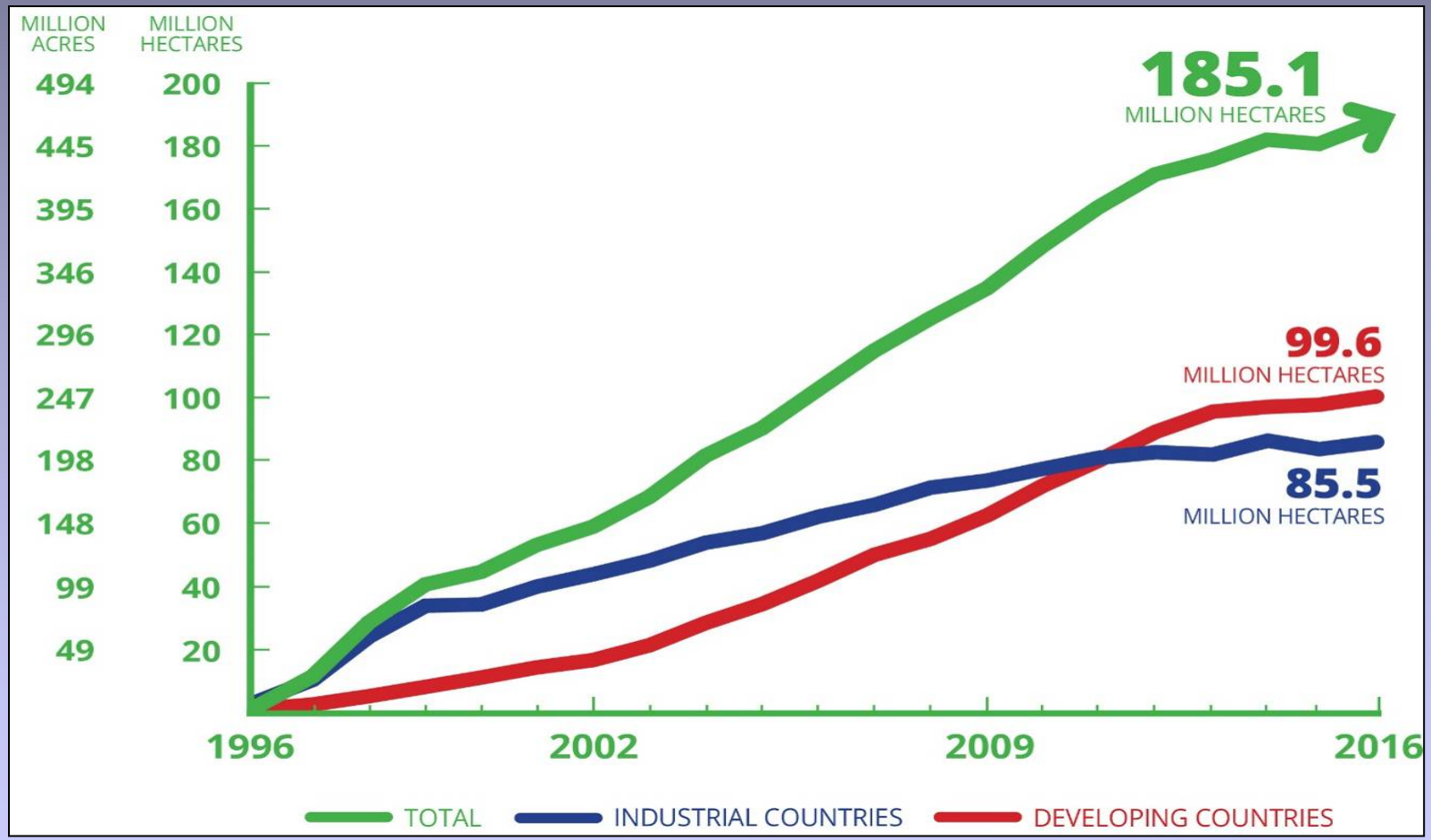
ucbiotech.org

There are only a few whole, Ge_n foods in the U.S market



Two more are just being introduced

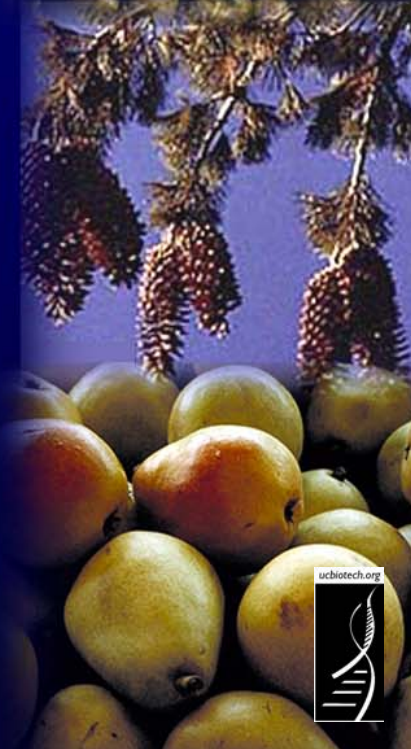
Despite the same limited U.S. crop and trait types, worldwide acreage is increasing in 19 developing, 7 developed countries



In 2016 ~18 million farmers in 26 countries planted 457M acres (>4X size of California) – 54% in developing countries; 41% stacked traits

James, C. 2016 http://www.isaaa.org/resources/publications/briefs/52/default.asp

WHAT'S IN THE PIPELINE?



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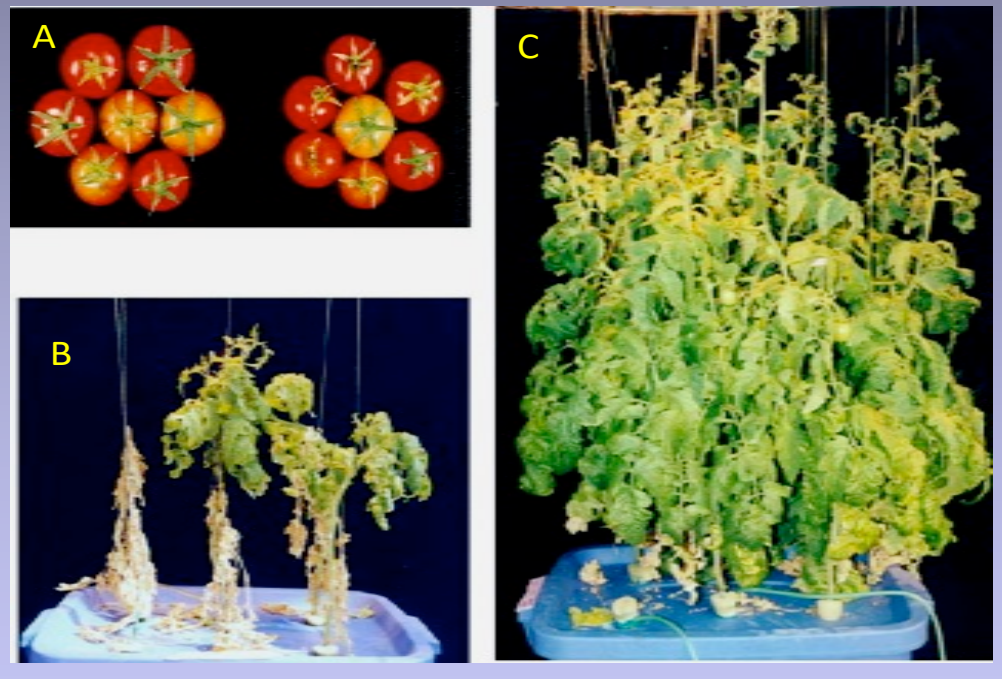
Salinity and Drought Tolerance - UC Davis



Wild type AtNHX1
200 mM NaCl (~1/2 sea water)

Salt-tolerance

Wild type IPT gene
15 days drought, 7 days re-watered

Drought-tolerance

Hanana M. 2011. Environ Rev 19: 121-140; Anjuman A et al. 2013 Mol. Biotechnol 54: 379-392

2013 GE potato field study – Ireland
Desiree potato variety, highly susceptible to late blight, engineered with gene from wild potato variety

amiga
Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems

eagasc
Agriculture and Food Development Authority

WAGENINGEN UR
For quality of life

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Chestnuts engineered with a wheat gene prevents cankers from forming; replanted with $104K raised through crowd funding campaign

http://www.newscientist.com/article/dn25644-american-chestnut

High anthocyanin purple GE tomatoes. Diets with 10% purple tomatoes increased lifespan of cancer-prone mice

Butelli et al. 2008. https://www.jic.ac.uk/staff/cathie-martin/purple-tomatoes.html

Engineered tobacco produces Zmapp, the drug used to treat ebola virus patients

SOURCE: "Bob Simon's final 60 Minutes: Grinding progress of ZMAPP Ebola GMO drug" , Genetic Literacy Project, 2/17/15. http://geneticliteracyproject.org/2015/02/17/bob-simons-final-60-minutes-grinding-progress-of-zmapp-ebola-gmo-drug/

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Cow horns are removed for safety of other cows and farmers but it is painful.

UCD researchers used editing to create precise mutations to create hornless dairy cows.

Carlson, et al. Nature Biotechnol. 2016.

How are these GMO/GE$_n$ Crops & Foods regulated? Three U.S. Agencies

USDA

- **Field testing**
  - **-Permits**
  - **-Notifications**
- **Determination of non-regulated status**

Plant pest?

FDA

- **Food safety**
- **Feed safety**

Danger to people?

EPA

- **Pesticidal plants**
  - **-tolerance exemption**
  - **-registrations**
- **Herbicide registration**

Risk to environment?

For variety created thru classical methods USDA requires data on:

- agronomic performance
- proximate analysis
  (protein, fat, CHO)
- antinutritive factors

Paperwork required for registration of conventional flax variety

A. McHughen,
UC Riverside

USDA requirements for GMO/GE_n varieties require that data plus…

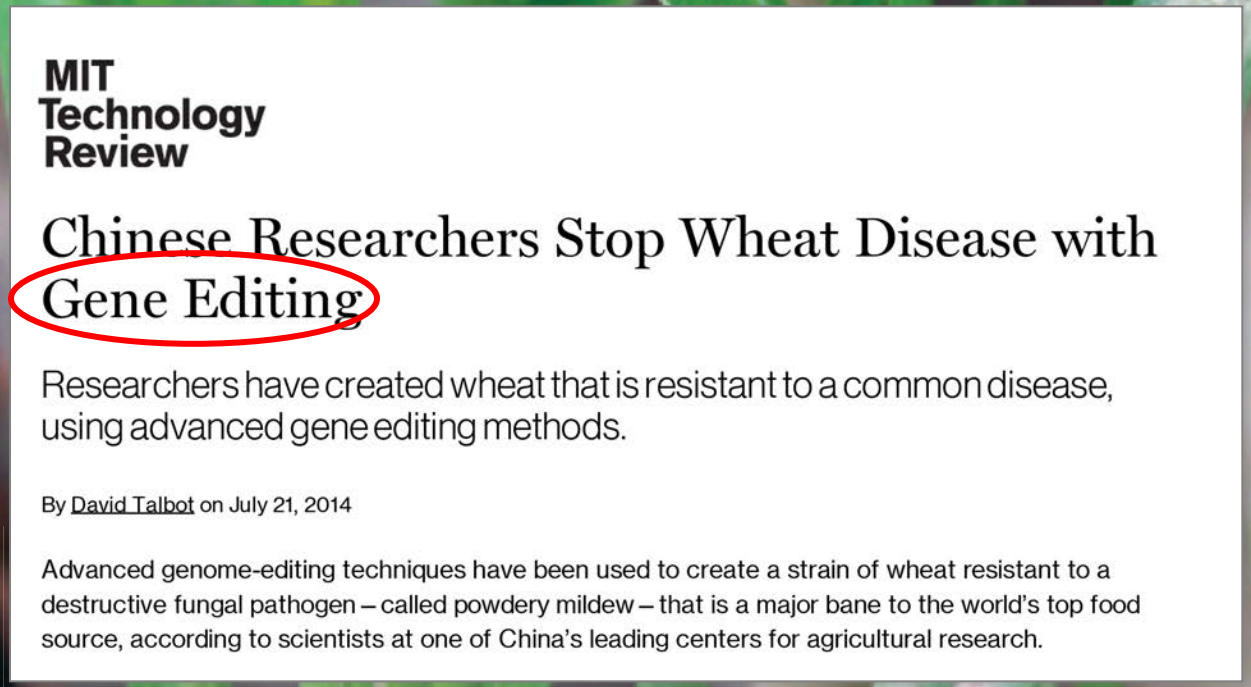
Paperwork required for registration of GE_n flax variety

A. McHughen
UC Riverside

- Molecular characterization of inserted DNA,
- Southern and restriction analyses
- PCR for several fragments,
- Various enzyme assays (ALS, NOS, NPT-II)
- Copy number of inserts
- Size of each fragment,
- Source of each fragment
- Utility of each fragment
- How fragments were recombined
- How construct was delivered into flax
- Biological activity of inserted DNA (genes)
- Quantitative analyses of novel proteins (western analyses)
- Temporal activity of inserted genes
- Spatial activity of inserted genes
- Complete amino acid analysis
- Detailed amino acid analysis for valine, leucine and isoleucine
- Toxicity
- Allergenicity
- Biological analysis:
- Pathogenicity to other organisms
- Dormancy,
- Outcrossing
- Potential for horizontal gene transfer
- Seed production
- Flowering time,
- Flower morphology
- Analysis of relatives
- Stability of inserted genes over seed generations
- Survivability in natural environment
- Survivability in agricultural environment in presence of herbicide
- Survivability in agricultural environment in absence of herbicide
- Interaction with other organisms- alterations to traditional relationships
- Interactions with other organisms- novel species
- Changes to persistence or invasiveness
- Any selective advantage to the GMO
- Any selective advantage to sexually compatible species
- Plan for containment and eradication in the event of escape

Not cheap! Industry estimates regulatory costs at $10-$20M per event! Beyond resources of academics & small companies

Now crops are being created using new genetic tools: gene editing using CRISPR technology



MIT Technology Review

Chinese Researchers Stop Wheat Disease with Gene Editing

Researchers have created wheat that is resistant to a common disease, using advanced gene editing methods.

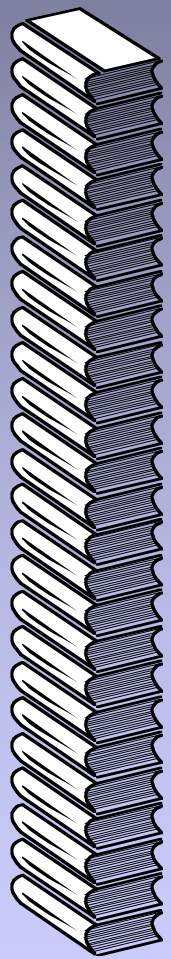
By David Talbot on July 21, 2014

Advanced genome-editing techniques have been used to create a strain of wheat resistant to a destructive fungal pathogen – called powdery mildew – that is a major bane to the world's top food source, according to scientists at one of China's leading centers for agricultural research.

Wheat resistant to powdery mildew created using new genome-editing techniques

SOURCE: "Chinese Researchers Stop Wheat Disease with Gene Editing", MIT Technology Review, July 21, 2014
http://www.technologyreview.com/news/529181/chinese-researchers-stop-wheat-disease-with-gene-editing/

New Genetic Method: Genome Editing-1



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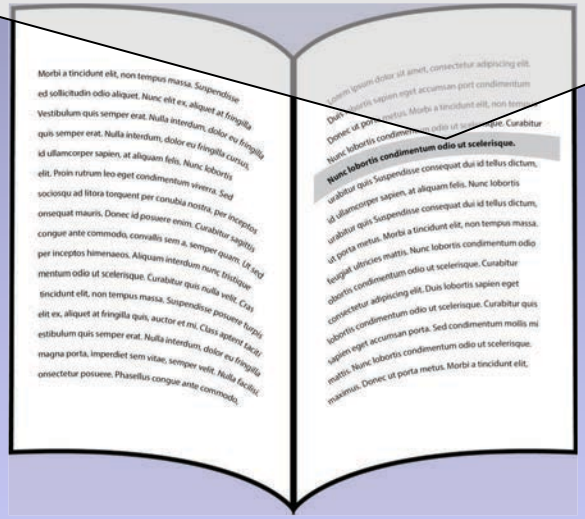
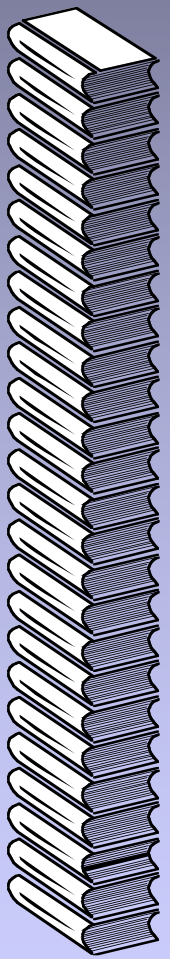
It is that new sentence which will be modified





Inserts specifically in genome

Find target text, cut out, paste in new modified text

1700 books (or 1.7 million pages)

These genome-edited plants are not GMO to USDA

1700 books (or 1.7 million pages)

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EXAMPLES of such edited products:

nature

NATURE | NEWS

Gene-edited CRISPR mushroom escapes US regulation

Jose A. Bernat Bacete/Getty Images

University

Dupont Develops Corn Using New CRISPR Technology

By KRISTOFOR HUSTED • APR 27, 2016

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LIZ WEST, FLICKR CREATIVE COMMONS

A new genetically engineered corn variety developed by one of the world's largest seed companies won't undergo the same review by regulators as other GMO crops.

Company

Capital Press

Agriculture Newspaper

Gene-edited camelina cleared by USDA

CRISPR-developed trait increases oil content

By MATEUSZ PERKOWSKI

Capital Press

A variety of camelina that's gene-edited to increase oil content can be grown without undergoing the USDA's regulatory process for biotech crops.

The agency has determined the camelina cultivar doesn't pose a plant pest risk, which means it's outside the USDA's regulatory jurisdiction over genetically modified organisms, or GMOs.

The crop's developer, Yield10 Bioscience, relied on a technology known as CRISPR to "knock out" a gene from camelina, eliminating a biological plant activity, thereby allowing it to produce more oil, said Kristi Snell, the company's chief science officer.

Camelina is an oilseed that's been studied as an alternative crop in Eastern Oregon and elsewhere in the arid West, since it's capable of surviving with minimal irrigation.

However, the crop isn't widely cultivated because it's currently not profitable enough for growers, said Snell. "You need to get the yield up to make it viable."

Yield10 Bioscience is examining the possibility of "stacking" the trait associated with increased oil content with

Turn to CAMELINA, Page 12

Wikimedia Commons

Camelina modified using CRISPR technology doesn't fall under the USDA's regulatory purview.

Company

Capital Press

Agriculture Newspaper

USDA won't regulate biotech wheat variety

Cultivar modified to 'knock out' mildew-susceptible gene

By MATEUSZ PERKOWSKI

Capital Press

A wheat variety rendered mildew-resistant through the targeted "knockout" of a gene can be commercialized without clearing USDA regulatory hurdles for biotech crops.

The agency's Animal and Plant Health Inspection Service has found that the cultivar doesn't fall under its jurisdiction for regulating genetically engineered crops, which is limited to possible plant pests and pathogens.

While the wheat was developed with genetic elements from disease-causing bacteria, they aren't contained in the crop and thus it's not subject to USDA's deregulatory process, which includes environmental analysis and public comment, according to APHIS.

Capital Press file

Wheat is shown in this file photo. The Animal and Plant Health Inspection Service has found that a new mildew resistant wheat cultivar doesn't fall under its jurisdiction for regulating genetically engineered crops.

Most biotech crops commonly grown in the U.S. have undergone deregulation, and in some cases, lawsuits over the adequacy of this process have delayed their commercialization.

Calyxt, a subsidiary of the biopharmaceutical company Cellectis, developed "MLO_KO" wheat with genetic sequences from bacteria and corn that remove a gene that suppresses the plant's defenses against powdery mildew.

The wheat's DNA is repaired during natural cellular processes and no foreign genetic material remains.

"It does not change the wheat's basic biology or produce a plant that would directly feed on, infect, parasitize, or contaminate plants, or adversely affect other organisms that are beneficial to plants," Calyxt said in a letter to APHIS.

The gene eliminated by Calyxt is involved in the plant's biological processes but the mildew fungus also relies on it to "trick" and penetrate the wheat crop, said Luc Mathis, the company's CEO.

Even without the gene, the wheat's biology remains unaffected due to other genes that perform duplicative roles, he said. "When you remove this function, the plant behaves normally."

Though the USDA has decided the wheat cultivar can be commercialized, Mathis said he doesn't expect the crop to be sold to farmers until 2022.

Calyxt must first conduct trials to ensure the trait is reliable in the field while simultaneously incorporating the mildew resistance into geographically suitable wheat varieties, he said.

Turn to WHEAT, Page 12

Company

In 2016/2017, USDA: can't regulate corn, mushroom, wheat, camelina made with genome editing because no DNA from plant pest, pathogen introduced, which is their regulatory hook.

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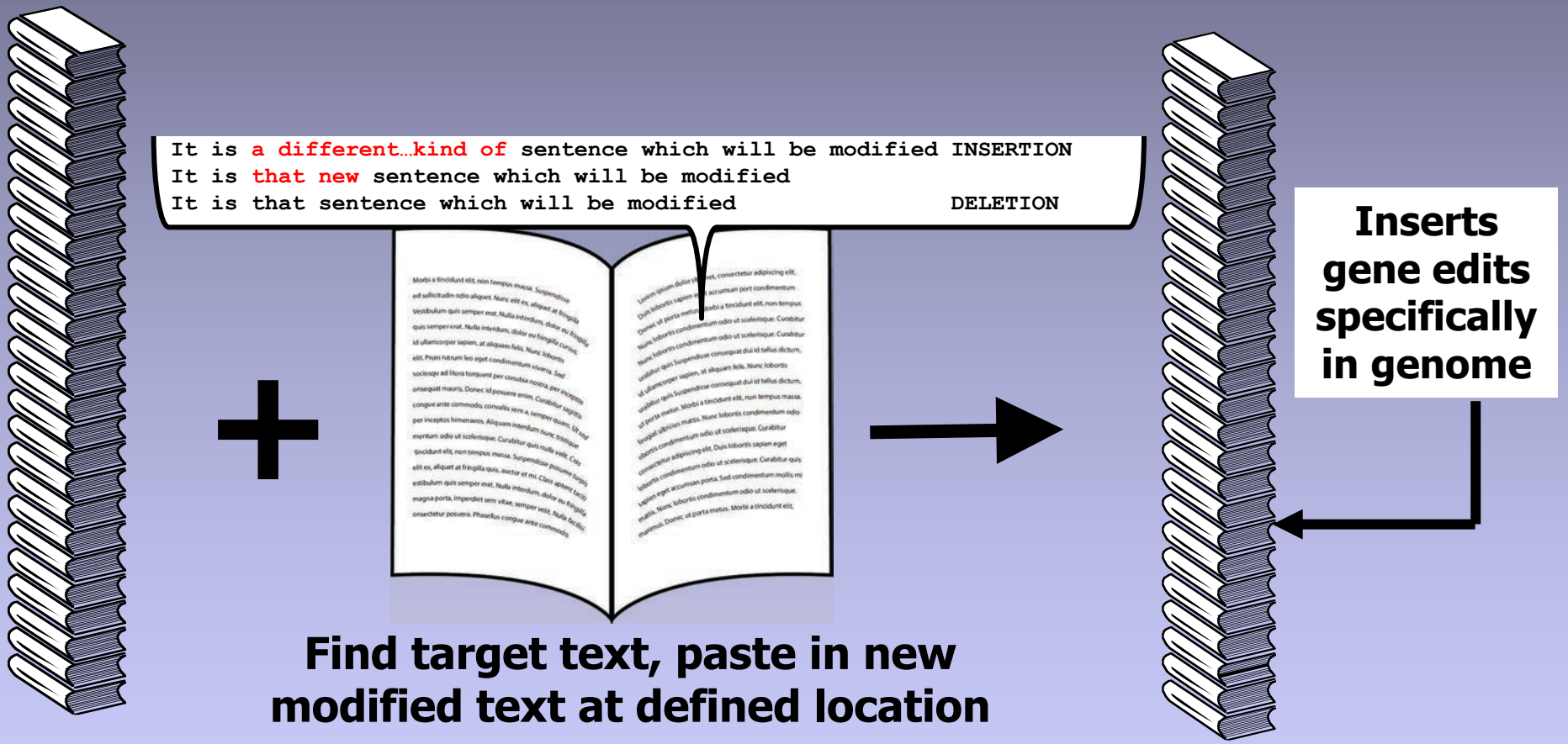
New Genetic Method: Genome Editing-2



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This type of genome editing *may or may not be* regulated as GE or GMO, depends on whether it contains certain types of DNA.

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

Technology, like editing, led to calls to revamp U.S. regulation

Genetically engineered crops that fly under the US regulatory radar

July 2, 2015: First step taken by White House Initiative to update 1986 Framework governing regulation.

Jan. 4, 2017 small attempt to update Coordinated Framework

Nov. 6, 2017: USDA has withdrawn a plan to overhaul how it regulates biotechnology products, such as genetically engineered crops; little detail about reason for change

Servick, K. "Trump's agriculture department reverses course on biotech rules" Science Nov. 6, 2017

suggesting that the use of technologies, such as null segregants, novel delivery systems,

importantly, that allows the participation of small companies and public sector institutions.

Camacho et al. 2014. Nature Biotech. 32:1087-1091

Are New Technologis for Food production Needed? Consider This As Food For Thought

- Nigeria: little over twice the size of California
- 75% more arable land than U.S.
- Five times less land per capita in Nigeria vs. U.S.
- In 2050, expected to be third most populous country in the world overtaking the U.S.

Source: United Nations http://www.un.org/apps/news/story.asp?NewsID=51526#.WRC5wFPytp8

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Also...Consider This As Food For Thought

If food waste were a country, it would rank behind only the US and China for greenhouse gas emissions.

And...production of wasted food uses 28% of the world's agricultural area.

So, we need to think about other changes as well.

Where to get more information on these topics?

THANKS

Questions?

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