

基因編輯技術及應用

Gene editing technologies and applications

賀端華

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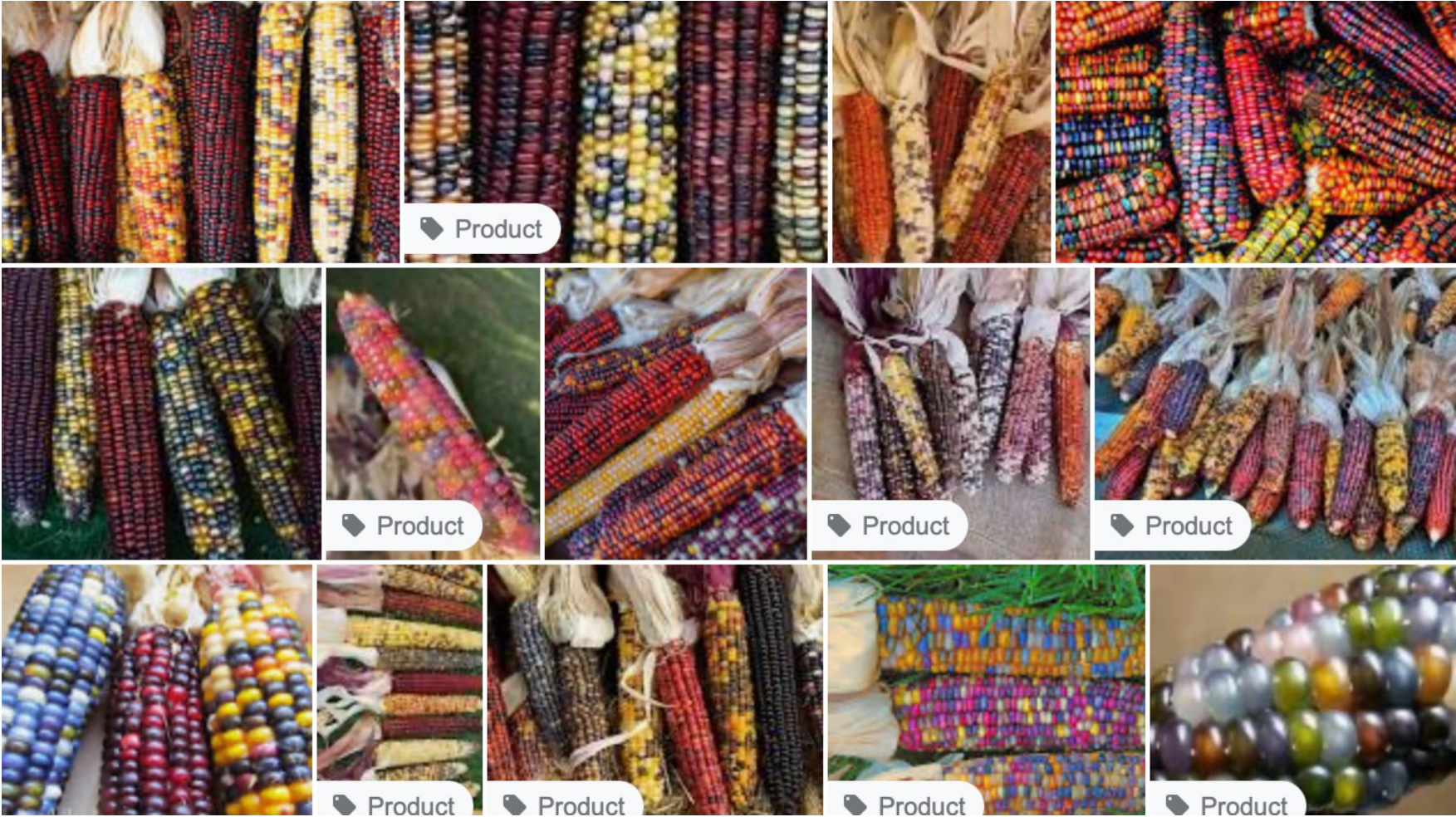
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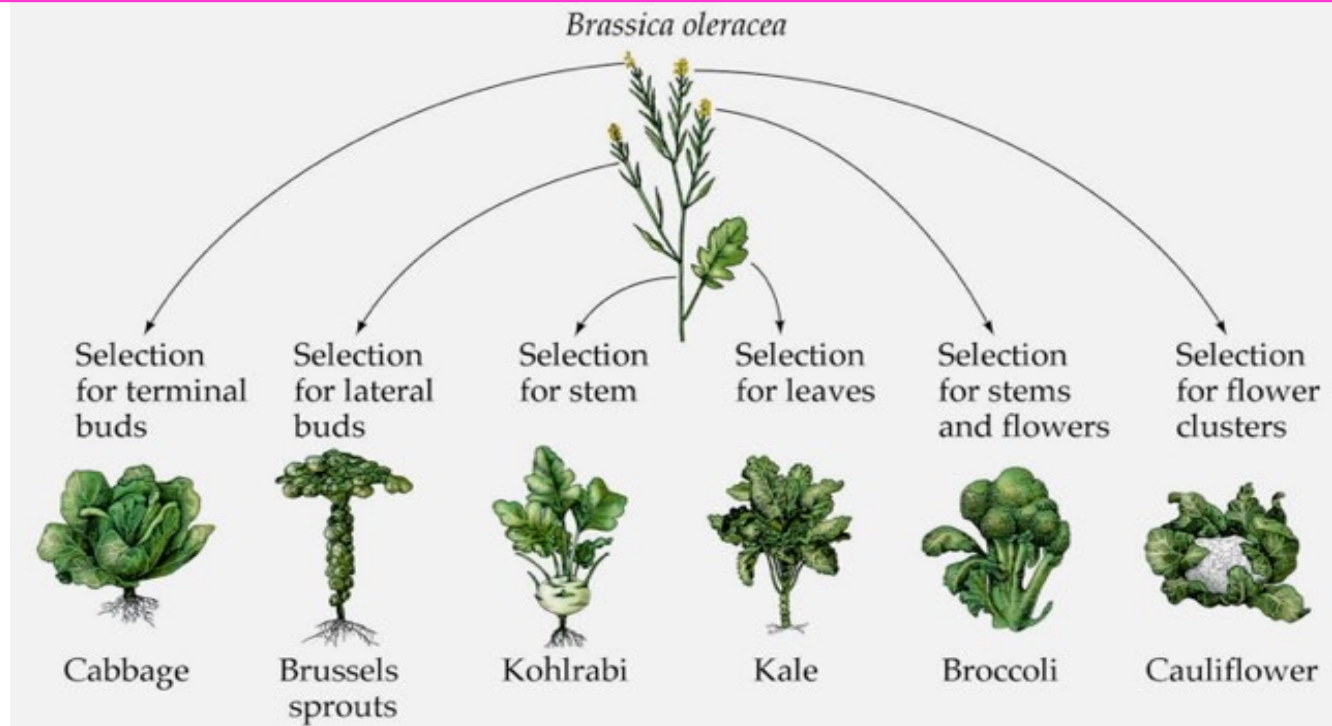
遺傳變異是演化及育種的動力

**Genetic variations are the driving force
for evolution, breeding and beyond**

There are many many interesting mutants around



Breeding of Brassica (十字花科) to different varieties to meet market demands



Cabbage



Brussel sprouts



Kohlrabi



Kale



Broccoli



Cauliflower

Gene mutations are the driving force for diversity and evolution.
基因突变是多樣性及演化的原動力。

Type of mutations: 突變的種類

- 1. Substitutions of nucleotide/amino acid 取代**
- 2. Insertions and deletions 插入或 去除**
- 3. Early stop/truncation/extension 截短或增長**
- 4. Duplications 複製**

Causes of mutations: 造成 突变的原因

- 1. Spontaneous 自然發生**
- 2. Induced by chemicals or radiation 化学或放射誘導**
- 3. Genetic modifications (GMO) 基因改造**
- 4. Genetic editing 基因編輯**

編輯的重要

下雨天留客天留我不留。

下雨天，留客天，留我不？留！

下雨天留客，天留，我不留！

下雨天，**不**留客，天留我不留！

A woman without her man is nothing.

A woman : without her, man is nothing.

A woman, without her man, is nothing.

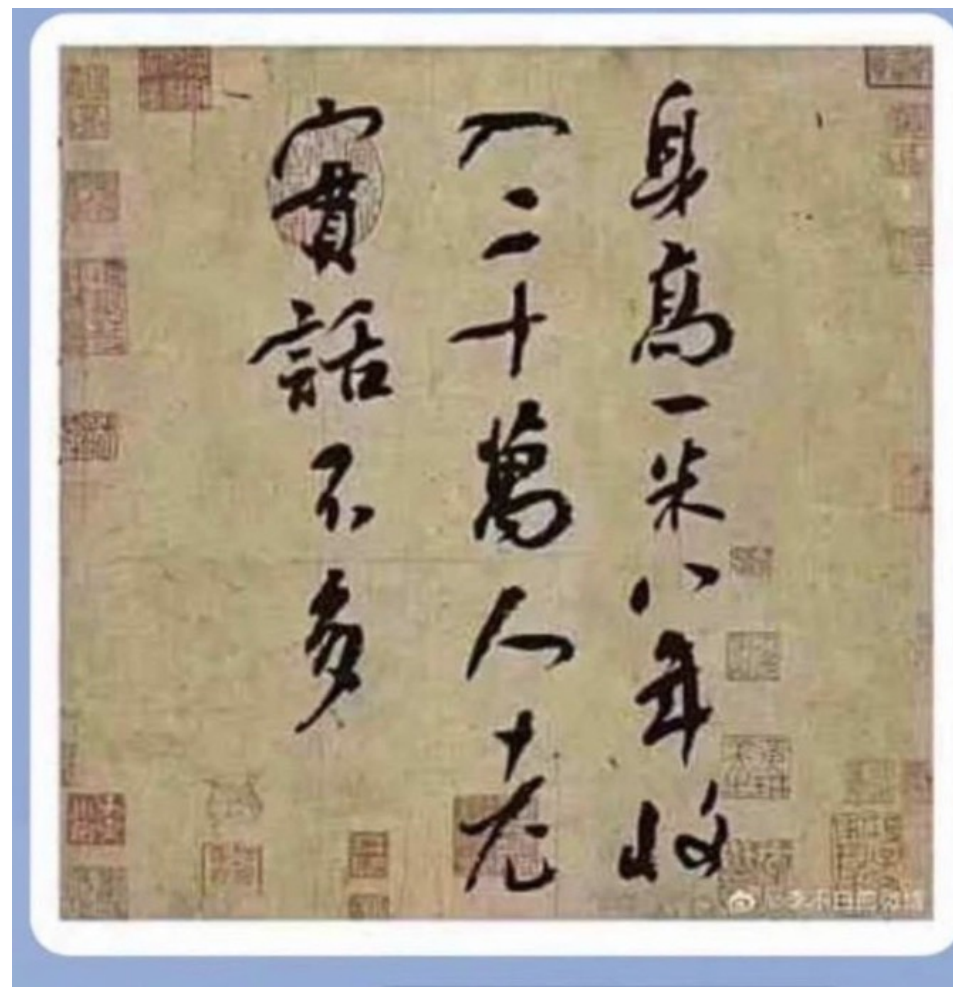
古人徵婚啟事：

"身高一米八，年收入二十萬，人老實，話不多。"

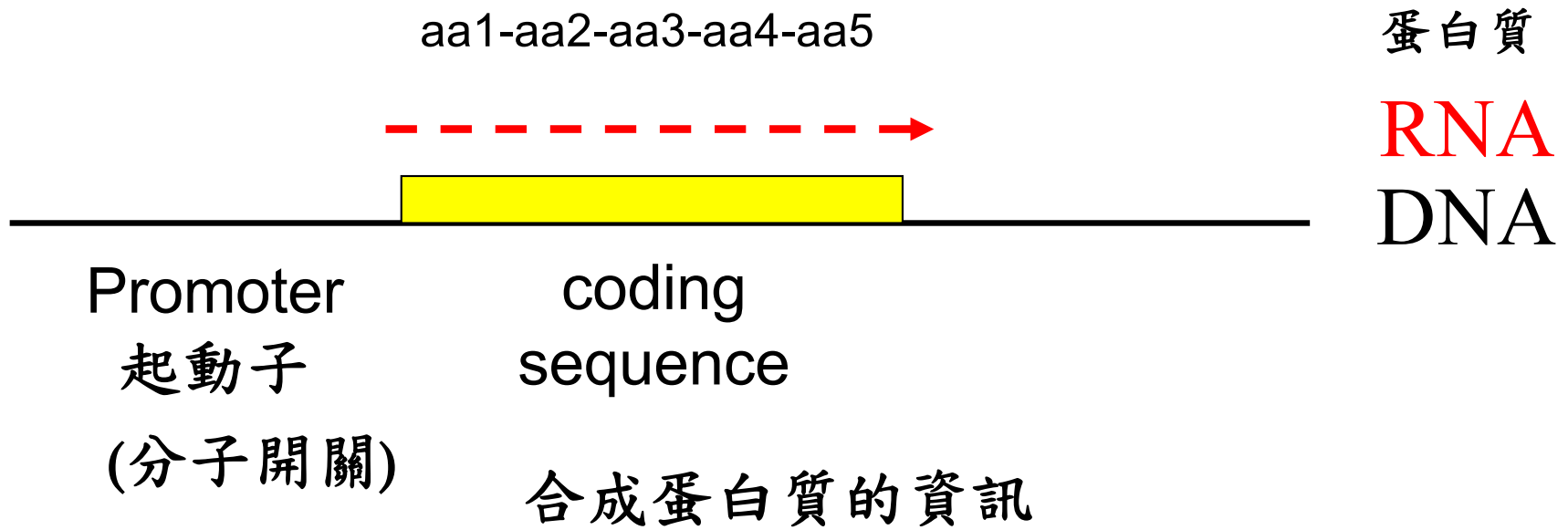
嫁後才知：

"身高一米，八年收入二十萬，人老，實話不多。"

原稿收藏於故宮



基因結構



起動子
(分子開關)

Promoter

合成蛋白質
的資訊

Coding region

————— **ATG TAT GGG TGG TAA** —————
Met Tyr Gly Trp Stop

Mutation:
Substitution

————— **ATG TAT TGG TGG TAA** —————
Met Tyr **Trp** Trp Stop

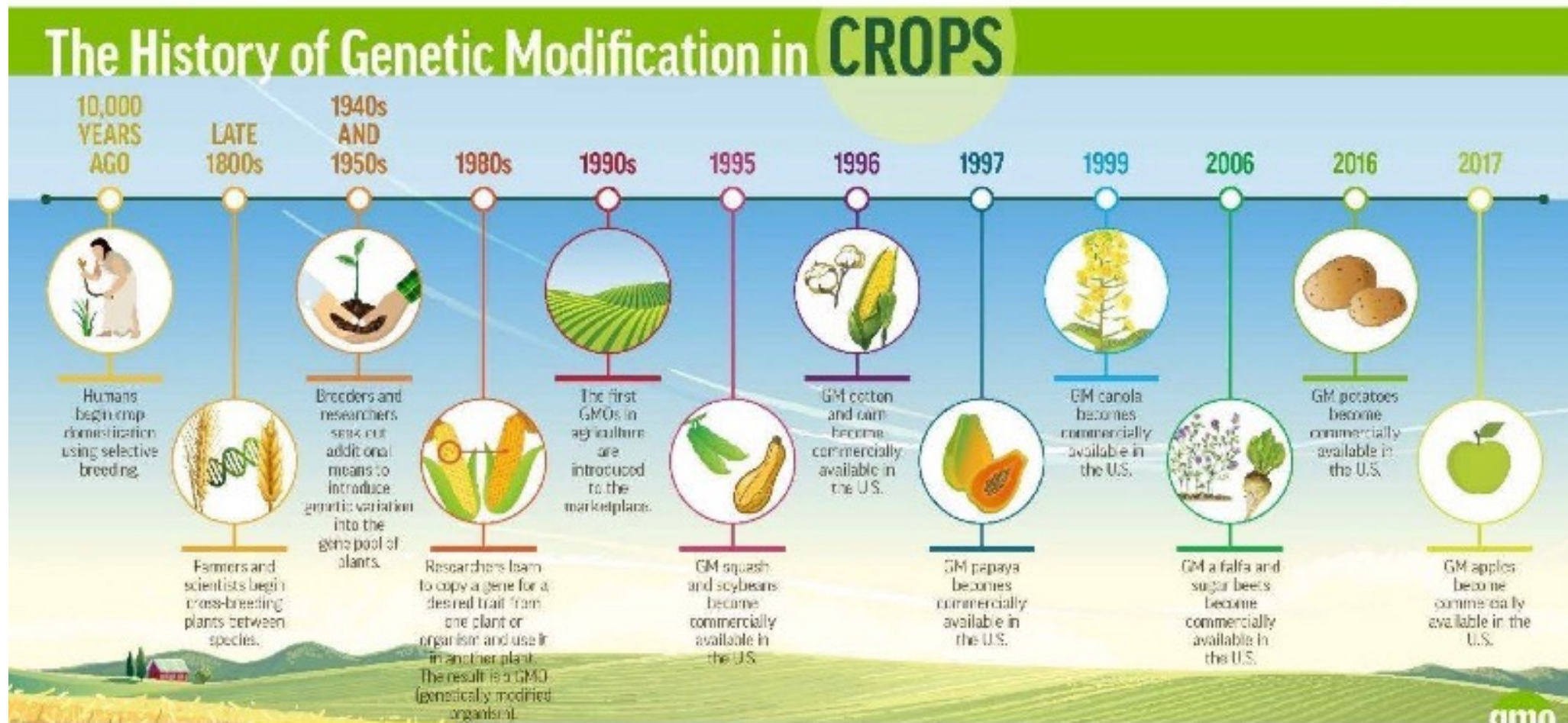
Mutation:
Early stop
Trucation

————— **ATG TAA GGG TGG TAA** —————
Met **Stop**

Mutation:
Insertion or deletion
Frame shift

基因改造及編輯只是最新的農業生物科技

The advancement in biotechnology facilitates molecular breeding



Domestication by selective breeding

Introduce genetic variation into the gene pool

1994, Calgene first marketed Flavr Savr - delayed-ripening tomato

Cross-breeding between species

Genetic modification (GM) by copy/past of gene from one to another organisms

Monsanto, GM squash and soybean on market

只需兩個主要基因的突變



玉米的老祖宗：
大蜀草



Téosite



Maïs primitif



Maïs actuel

雜交玉米的故事

傳統育種造成大量基因的移轉



B.



化學、物理學育種：化學藥劑、放射線使基因突變
Breeding by chemical or physical mutagenesis approaches



Chemical mutagens



Radiation



結果有好有壞！
The result may not always be desirable.



突變前

突變後



BEFORE & AFTER

- 
- A close-up photograph of a green wheat ear, showing the individual grains and the awns. The background is a soft, out-of-focus green field.
- 現代農業生技的突破
 - 目前已使用的GMO產品
 - 未來GMO的產品

合成蛋白質的資訊在不同生物中可以通用 (Genetic codes are universal)

起動子
(分子開關)
Promoter

合成蛋白質
的資訊
Coding region

動物

————— ATG TAT GGG TGG TAA —————
Met Tyr Gly Trp Stop

植物

————— ATG TAT GGG TGG TAA —————
Met Tyr Gly Trp Stop

微生物

————— ATG TAT GGG TGG TAA —————
Met Tyr Gly Trp Stop

細菌
起動子

人類胰島素

我們是否有使用過基因改造的食品或藥品?

- 許多糖尿病患者都需要使用人類胰島素來控制病情。
- Q: 但人類胰島素是如何產製的?
- A: 將人類胰島素的基因在細菌內大量表現。



沒有牛肉的 大漢堡



Ingredients:

1. **Leghemoglobin produced by engineered yeast**
(基改酵母菌產製的豆類血紅素)
1. Potato/soybean proteins
2. Coconut oil/canola oil

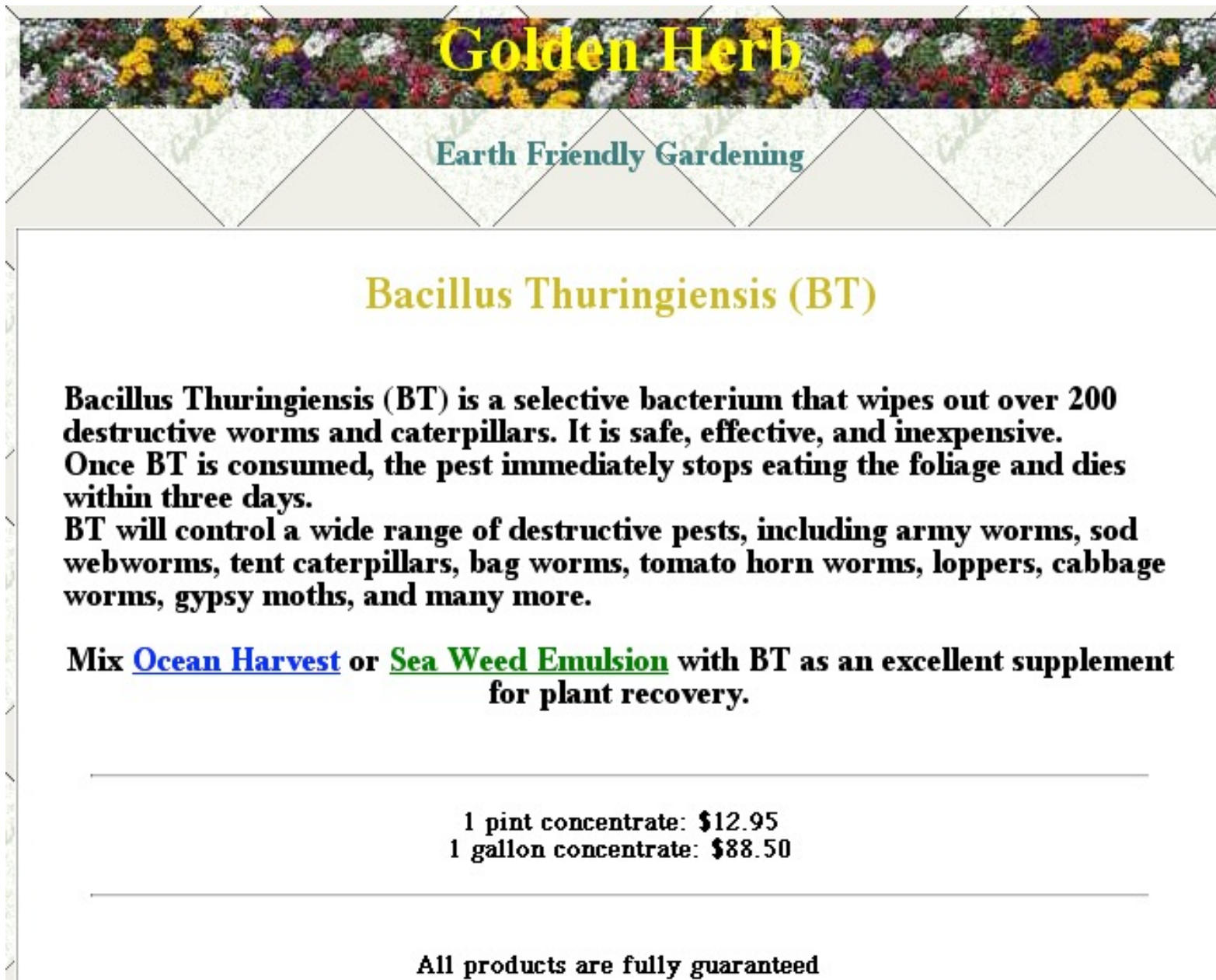
NOW AVAILABLE NATIONWIDE!
Limited time only at participating restaurants. Patty made from plants.
TM & © 2019 Burger King Corporation. Impossible is a trademark of Impossible Foods Inc. Used under license.

基改作物實例:

Bt corn (Insect-resistant corn): 抗蟲玉米

蘇力菌原本是有機農業界的寵兒：

Bt bacteria sold as an natural pesticide for gardening ...



Golden Herb
Earth Friendly Gardening

Bacillus Thuringiensis (BT)

Bacillus Thuringiensis (BT) is a selective bacterium that wipes out over 200 destructive worms and caterpillars. It is safe, effective, and inexpensive. Once BT is consumed, the pest immediately stops eating the foliage and dies within three days.

BT will control a wide range of destructive pests, including army worms, sod webworms, tent caterpillars, bag worms, tomato horn worms, loppers, cabbage worms, gypsy moths, and many more.

Mix [Ocean Harvest](#) or [Sea Weed Emulsion](#) with BT as an excellent supplement for plant recovery.

1 pint concentrate: \$12.95
1 gallon concentrate: \$88.50

All products are fully guaranteed

Bt protein used extensively in organic farming for the past thirty years



Applied as a foliar spray

Breaks down in the environment within a few days

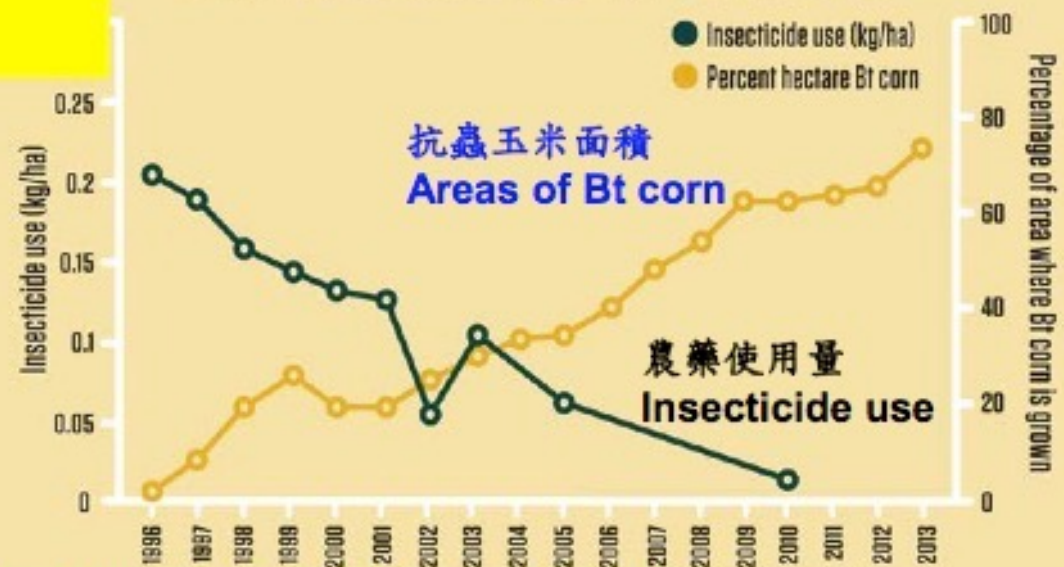
Courtesy USDA

基因改良育種：減少殺蟲劑使用
Breeding by GE: to reduce the use of insecticides



- Farmer's and consumer's health
- Environmental protection
- Production cost

Bt corn uptake and insecticide use in U.S. corn fields



- 
- 現代農業生技的突破
 - 目前已使用的GMO產品
 - 正在發展中的GMO產品

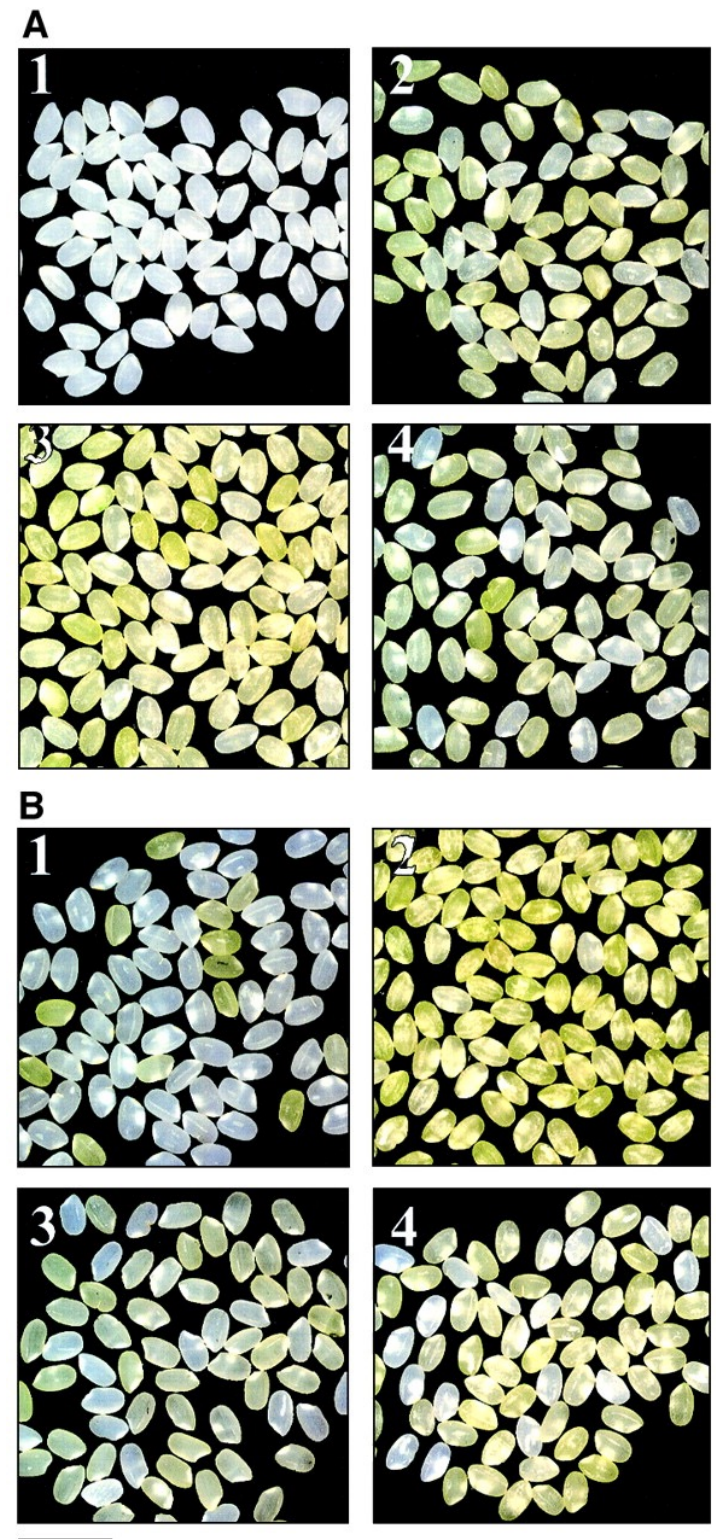
如何以基因工程的技術 來改進植物的營養成分

黃金米

Golden Rice



At least 1 million children, weakened by vitamin-A deficiency, die every year and an additional 350,000 go blind. ... but protesters believe such GMO are bad for us and our planet.

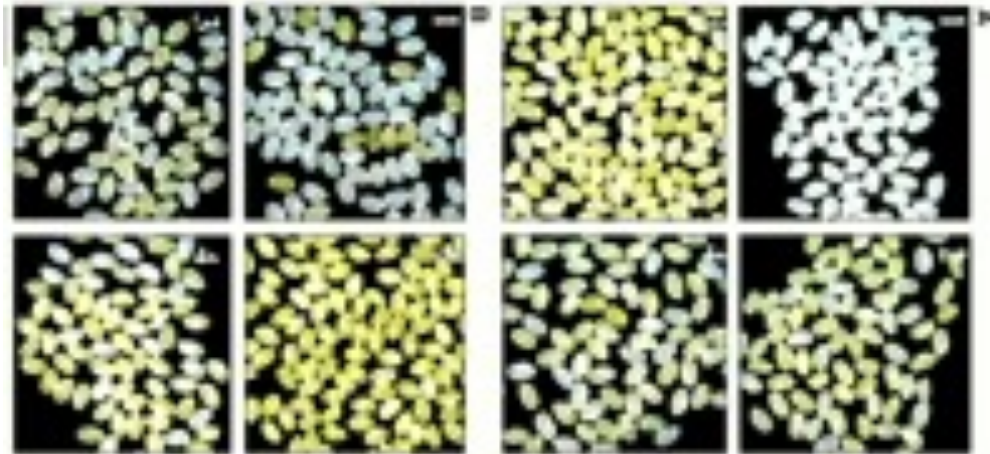
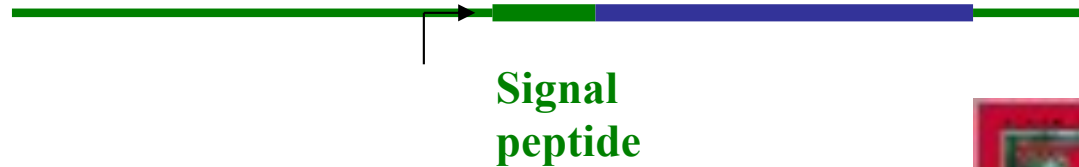


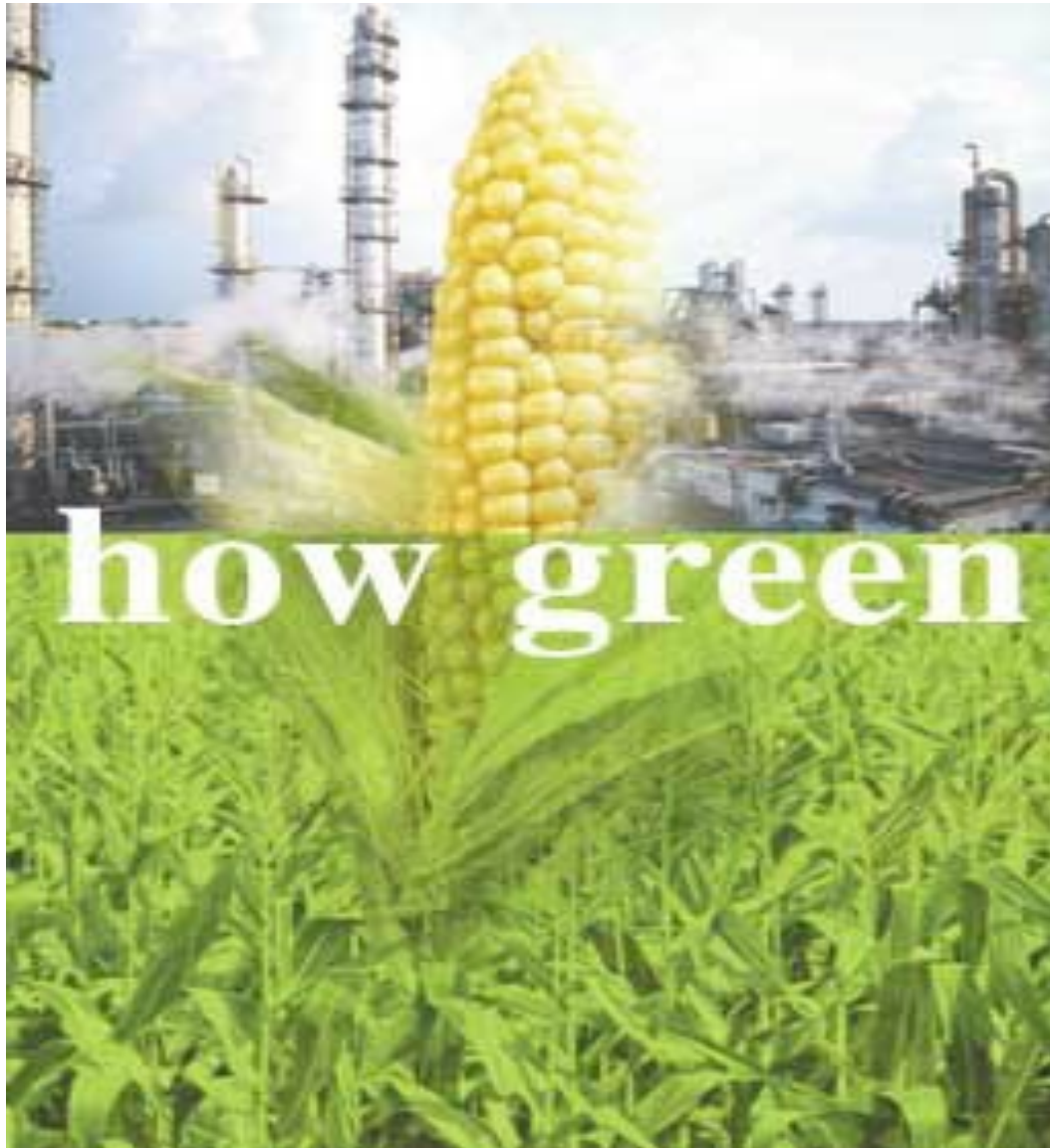
如何以基因工程的技術來改進植物的營養成分

Golden Rice (黃金米)

Promoter of a storage protein gene

Coding region of bacterial or daffodil phytoene synthase





以植物產製工業用
之塑膠原料

are green plastics?

Plant sugars(糖)



**Bacterial
enzymes**



Plastics(塑料)

PHA (polyhydroxyalkanoate)

PLA (polylactide)

以植物來產製動物用 抗體

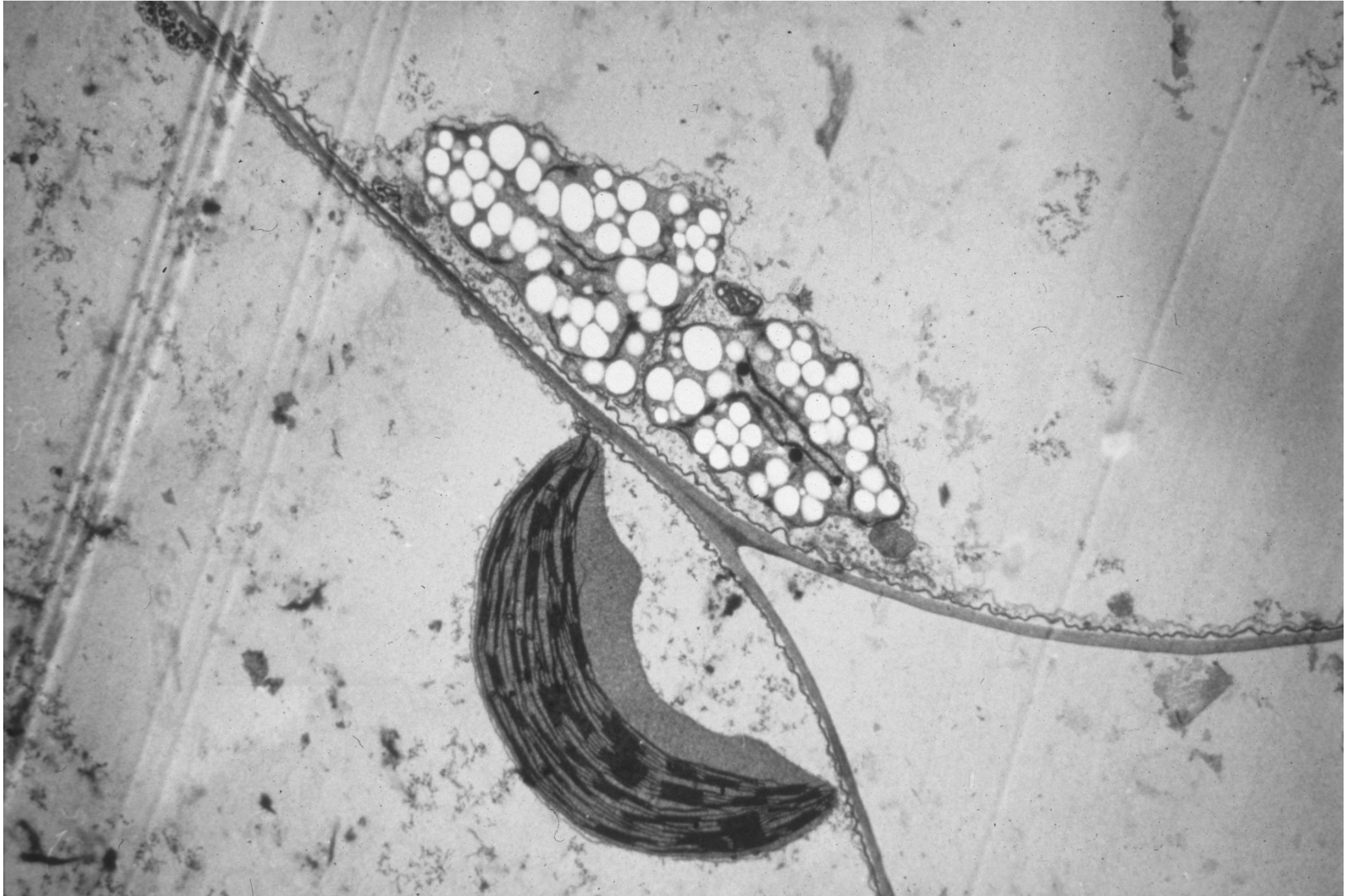
Animal proteins
(antibodies)
produced in
transgenic **plants**

Plant
promoter

Coding region
of antibody gene



以植物產製工業用之塑膠原料



植物產製的塑膠是比較符合環保需求的



control

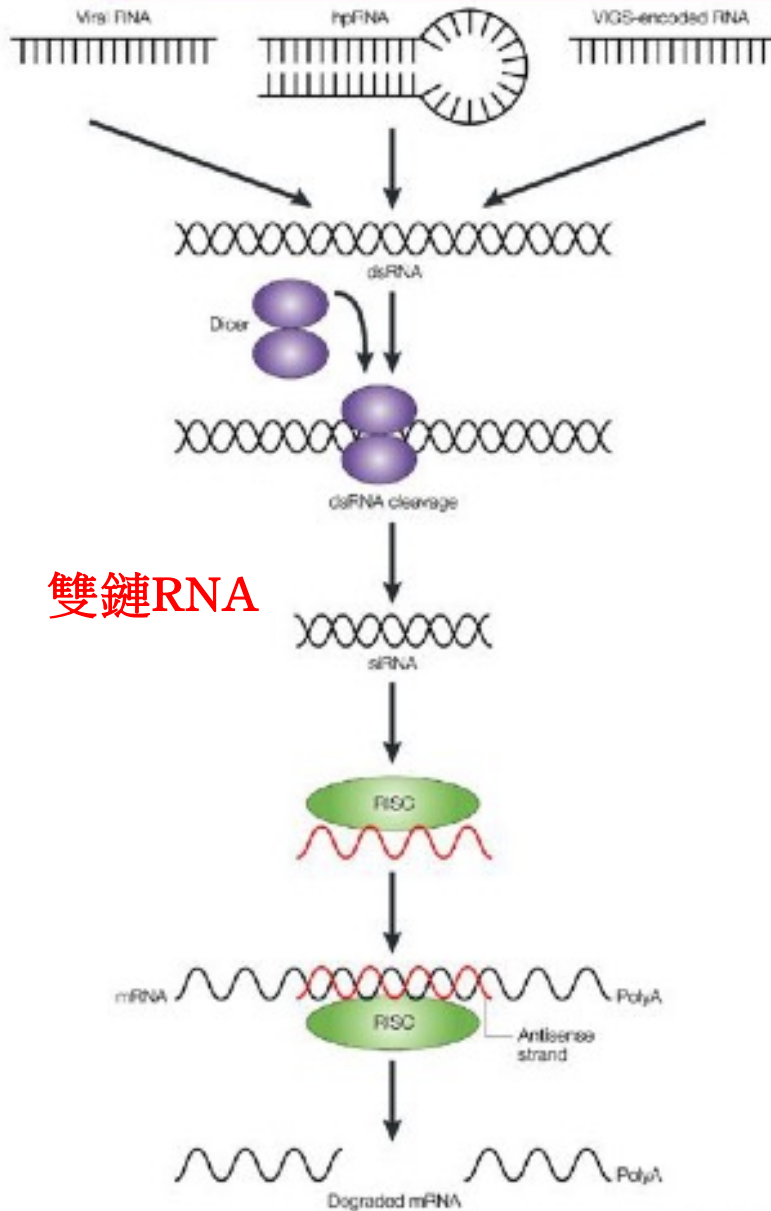
3 months in compost

9 months in compost

The GMO technology is the “addition” of new genes, but how about taking out or reducing the function of an existing gene?

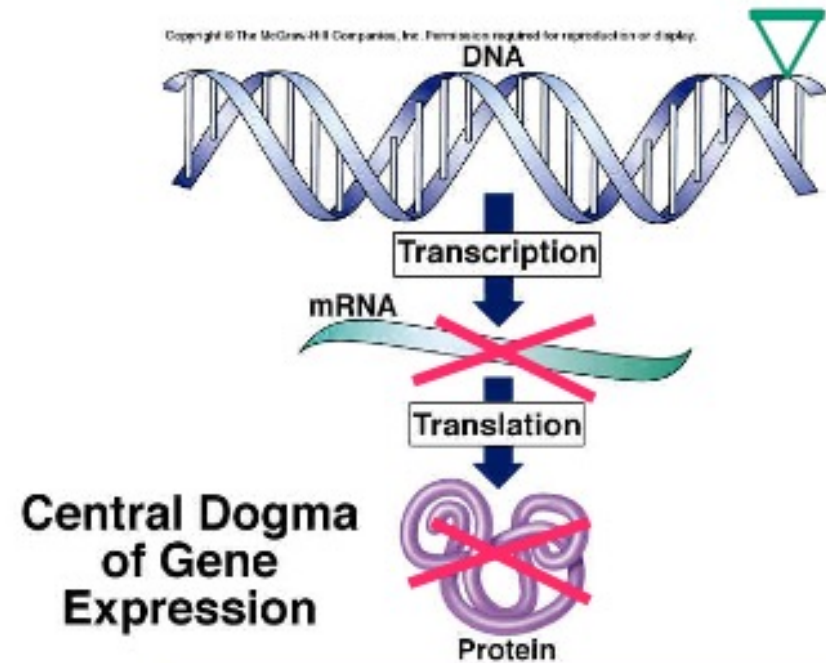
所謂的基因改造技術通常是增加一個或多個外源基因，但是如何可以改變
內源基因的功能呢？

基因靜默法， Gene silencing by RNA interference approach



Insert double strand (ds) RNA of target gene

mRNA干擾，讓不需要的蛋白質不要產生



No foreign protein is produced

RNA干擾是指一種分子生物學上由雙鏈RNA誘發的基因沉默現象

Arctic apple developed in B.C. approved for sale in U.S.

Genetically modified fruit inhibits enzyme that causes fruit to turn brown

CBC News Posted: Feb 13, 2015 10:43 AM PT | Last Updated: Feb 13, 2015 11:06 AM PT

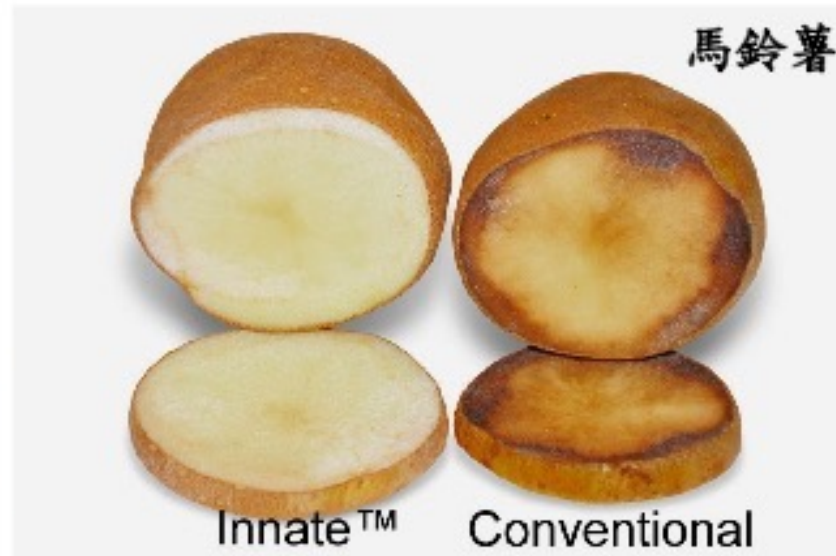
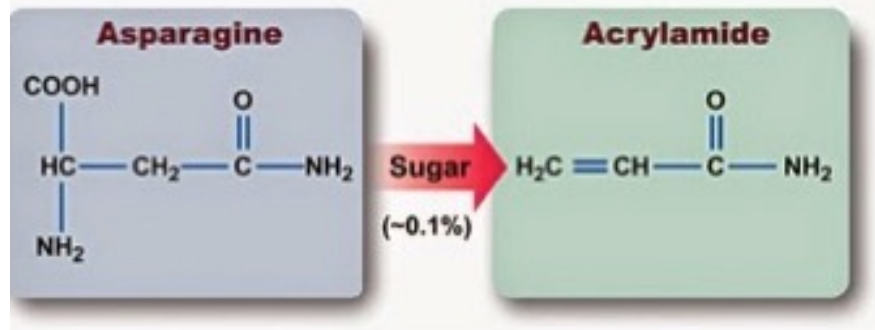


A company in B.C.'s Okanagan region is celebrating today after its genetically modified, **non-browning Arctic Apples** was approved for deregulation in the U.S.

The Arctic apple doesn't oxidize — or turn brown — because its developers have figured out how to adjust the growing process to inhibit the browning enzyme

加拿大的一家小公司Okanagan Specialty Fruits 利用基因靜默法抑制蘋果多酚氧化酶 (Polyphenol oxidase)的含量，所生產切開不會褐化的新品種稱為「北極蘋果」(Arctic apple)，最近也在美國及加拿大獲准商業化，相當受到餐飲業的歡迎，已於2017年上市，品牌有「Arctic Fuji」，「Arctic Granny」及「Arctic Golden」等。

GE potato that does brown when cut or fried, nor does it make acrylamide



Acrylamide is formed when **Asparagine** is heated to high temperatures (like when frying) in the presence of some **sugars**. Less Asparagine and sugars means less Acrylamide.



澱粉經過高溫燒烤或油炸後，所含**還原糖**會與一種氨基酸—**asparagine**作用而產生**acrylamide**，過量食用可能致癌及傷害神經。因此，如何降低澱粉類食品中**asparagine**的含量，是作物育種上很重要的一項工作，尤其在製作麵包的小麥及薯條、薯片的馬鈴薯。

美國愛達荷州**Simplot**公司「天生馬鈴薯」(**Innate potato**)降低 **asparagine**及糖含量

- Gene editing is much more versatile and efficient than gene silencing
- 基因编辑比基因静默更有弹性及效率

編輯的彈性運用

下雨天，留客天，留我不？留！

下雨天留客，天留，我不留！

下雨天，**不**留客，天留我**還**是**不**留！

不下雨天，**不**留客，天**不**留但我**還**是**要**留！

A woman without her man is nothing.

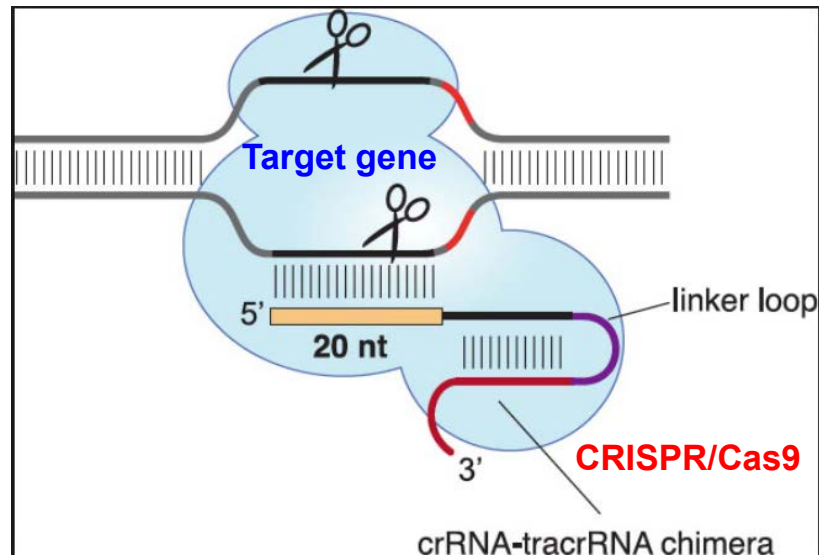
A woman : without her, man is nothing.

A woman : without her, man **can still survive .**

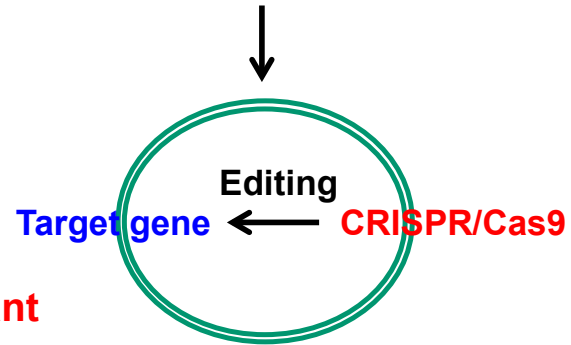
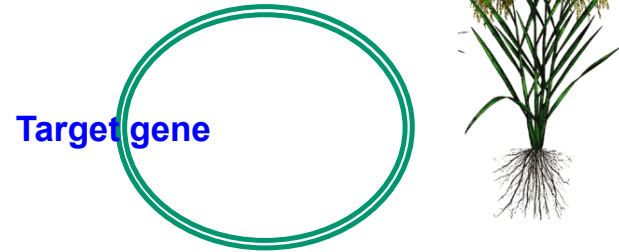
A woman, without her man, is nothing.

A woman, without her man, is **everything.**

CRISPR/Cas9 gene editing system



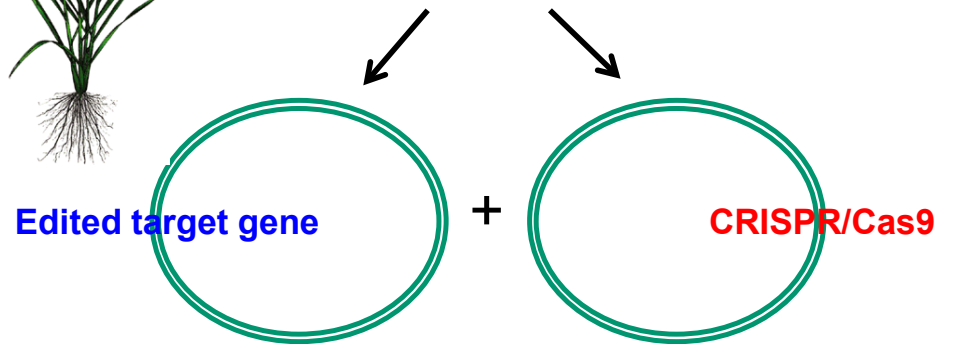
Disease sensitive



Disease resistant



Segregation



- Gene knockout is easy.
- Multi-gene editing is possible.
- DNA insertion is still uneasy.
- Weak gene expression could be a problem.

美國農業部已經公告某些基因編輯產品將不需管制

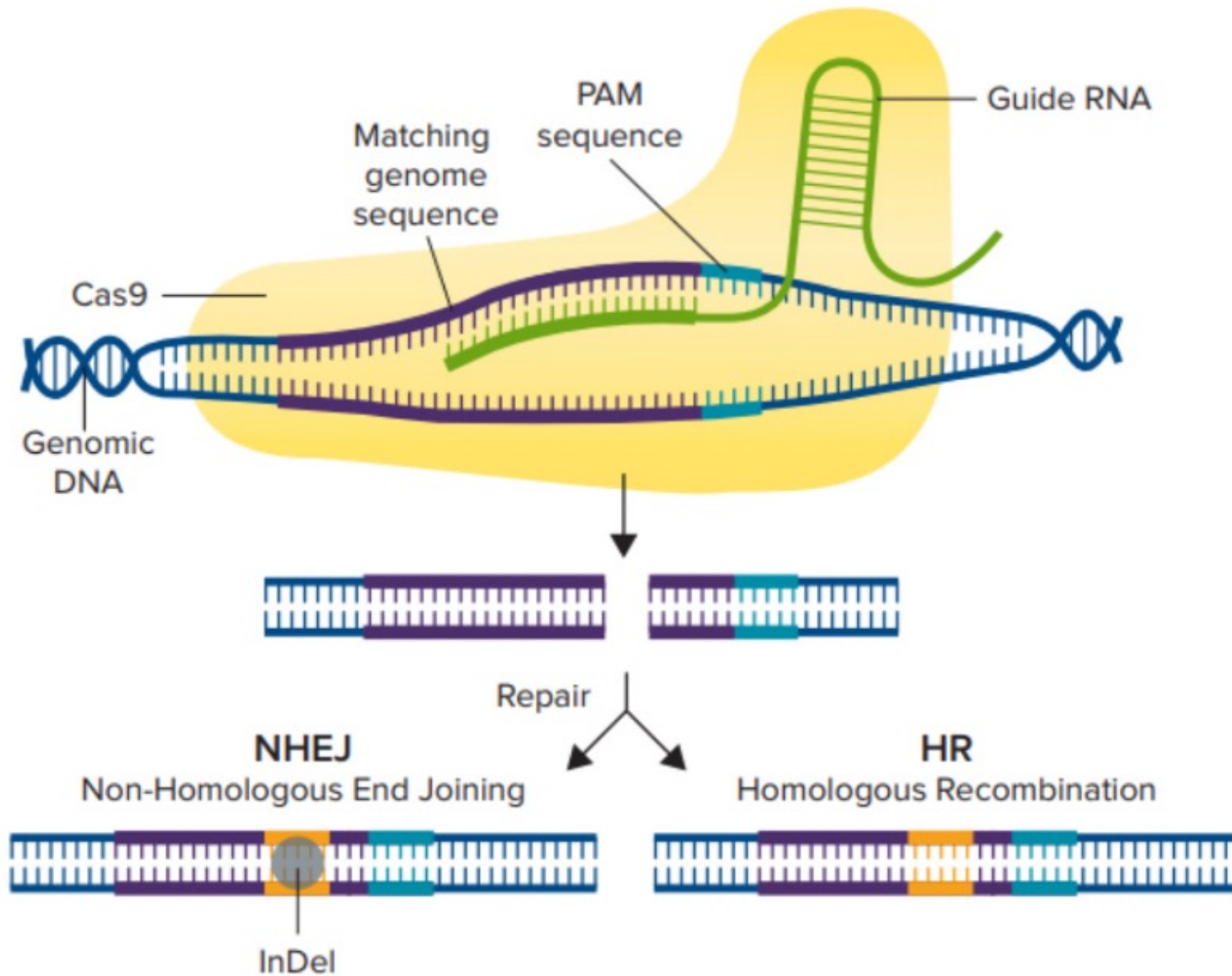
CRISPR stands

for Clustered Regularly Interspaced Short Palindromic Repeats. It is a component of bacterial immune systems that can cut DNA, and has been repurposed as a gene editing tool. It acts as a precise pair of molecular scissors that can cut a target DNA sequence, directed by a customizable guide.

The system is made up of two key parts: a CRISPR-associated (Cas) nuclease, which binds and cuts DNA, and a guide RNA sequence (gRNA), which directs the Cas nuclease to its target. It was discovered in bacterial immune systems, where it cuts the DNA of invading viruses, called bacteriophage, and disables them. Once the molecular mechanism for its DNA-cleaving ability was discovered, it was quickly developed as a tool for editing genomes.

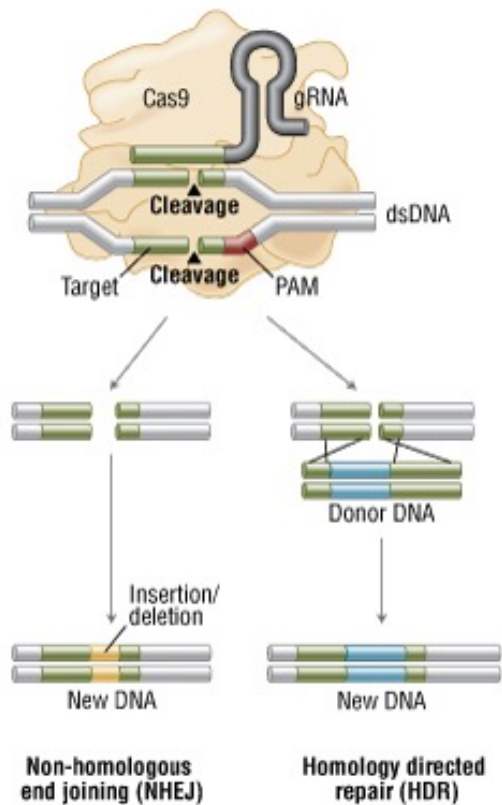
CRISPR is important because it allows scientists to rewrite the genetic code in almost any organism. It is simpler, cheaper, and more precise than previous gene editing techniques. Moreover, it has a range of real-world applications, including curing genetic disease and creating drought-resistant crops.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

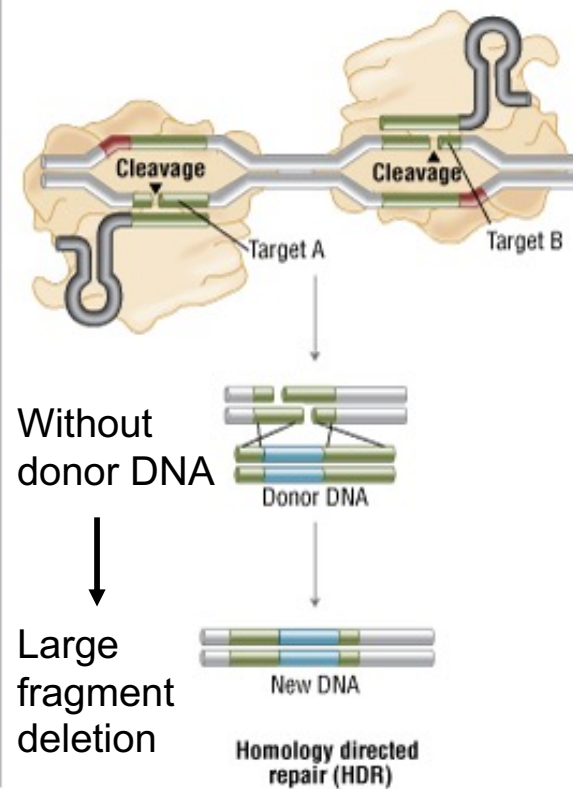


CRISPR/Cas9 System Applications

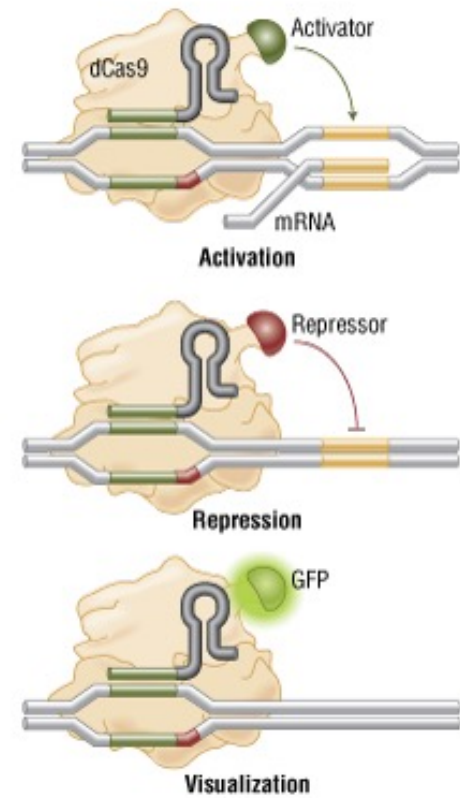
A. Genome Engineering With Cas9 Nuclease



B. Genome Engineering By Double Nicking With Paired Cas9 Nickases



C. Localization With Defective Cas9 Nuclease



CRISPR Design Tool — design CRISPR/Cas guide RNA sequence

select target sequence(s) from the output list — choose highly specific target

Sequence name: Marchantia FCA
 PAM sequence: NGG
 Specificity check: .1
 Time: 2023-03-08 13:26:36

- Highlighted target positions (e.g., 45 - 67) indicate sequences that are highly specific and have fewer off-target hits.
- Target sequences with '0' in '20mer+PAM' (in number of target sites column) are shown in gray. Such sequences may possibly span over exon-exon junctions, so avoid using these.
- Target sequences with TTTTs are also shown in gray. Avoid TTTTs in gRNA vectors with pol III promoter.

show highly specific target only

Show entries

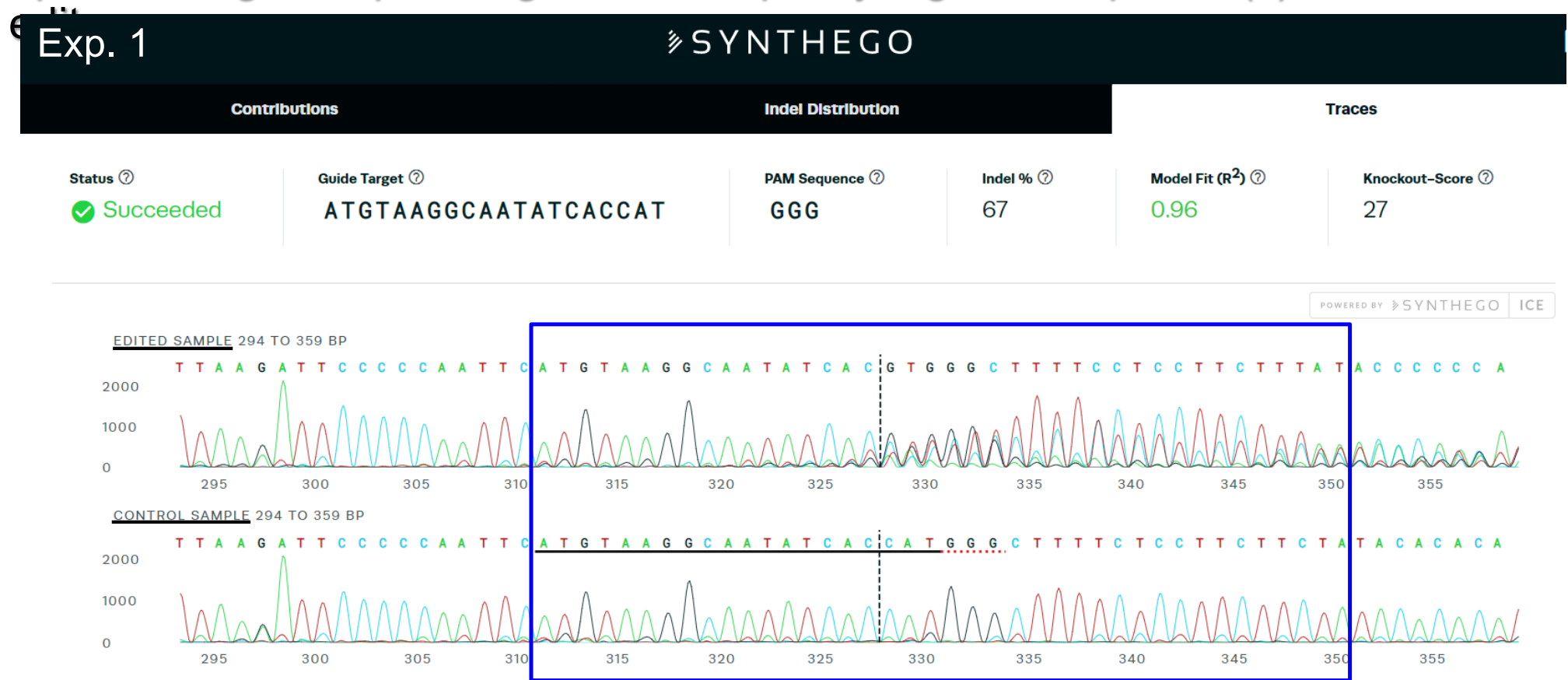
Search:

position		target sequence	sequence information				number of target sites ?		
start - end	+ -	20mer+ PAM (total 23mer)	GC% of 20mer	Tm of 20mer	TTTT in 20mer	restriction sites	20mer +PAM	12mer +PAM	8mer +PAM
10 - 32	-	cca cctctgctgcagcaacctta [gRNA]	60.00 %	78.84 °C	-	PstI SfcI	1 [detail]	7 [detail]	795 [detail]
13 - 35	-	cct ctgctgcagcaaccttacgc [gRNA]	65.00 %	80.23 °C	-	PstI SfcI	1 [detail]	4 [detail]	815 [detail]
27 - 49	-	ccc ttacgctatgatgcaacagc [gRNA]	45.00 %	70.20 °C	-		1 [detail]	2 [detail]	81 [detail]
28 - 50	-	cct tacgctatgatgcaacagca [gRNA]	45.00 %	71.20 °C	-		1 [detail]	1 [detail]	74 [detail]
61 - 83	-	ccg cagcatctgtctcagcaaca [gRNA]	50.00 %	71.55 °C	-		1 [detail]	1 [detail]	387 [detail]
69 - 91	+	tctgtctcagcaacagccgc agg [gRNA]	60.00 %	77.07 °C	-		1 [detail]	2 [detail]	150 [detail]
70 - 92	+	ctgtctcagcaacagccgc ggg [gRNA]	60.00 %	77.11 °C	-		1 [detail]	1 [detail]	185 [detail]
85 - 107	-	ccg cagggtaacaagctcccca [gRNA]	60.00 %	78.09 °C	-	HincII	1 [detail]	1 [detail]	129 [detail]
103 - 125	-	ccc catcagcatcagcagcctca [gRNA]	55.00 %	74.73 °C	-		1 [detail]	10 [detail]	579 [detail]
104 - 126	-	ccc atcagcatcagcagcctcag [gRNA]	55.00 %	75.17 °C	-		1 [detail]	5 [detail]	275 [detail]
105 - 127	-	cca tcagcatcagcagcctcagc [gRNA]	60.00 %	77.71 °C	-		1 [detail]	15 [detail]	499 [detail]
121 - 143	-	cct cagcagcagccccaagctcc [gRNA]	70.00 %	82.97 °C	-		1 [detail]	9 [detail]	1344 [detail]
124 - 146	+	cagcagcagccccaagctcc tgg [gRNA]	70.00 %	82.97 °C	-		1 [detail]	1 [detail]	372 [detail]
125 - 147	+	agcagcagccccaagctcct ggg [gRNA]	65.00 %	83.53 °C	-		1 [detail]	2 [detail]	377 [detail]
133 - 155	-	ccc caagctcctgggatttatga [gRNA]	45.00 %	70.10 °C	-		1 [detail]	2 [detail]	430 [detail]
134 - 156	-	ccc aagctcctgggatttatgat [gRNA]	40.00 %	69.35 °C	-		1 [detail]	2 [detail]	341 [detail]
135 - 157	-	cca agctcctgggatttatgatc [gRNA]	45.00 %	70.44 °C	-		1 [detail]	5 [detail]	432 [detail]
142 - 164	-	cct gggatttatgatcagcagca [gRNA]	45.00 %	70.25 °C	-	BclI	1 [detail]	3 [detail]	108 [detail]
147 - 169	+	gatttatgatcagcagcagc agg [gRNA]	45.00 %	68.91 °C	-	BclI	1 [detail]	28 [detail]	2349 [detail]
148 - 170	+	atztatgatcagcagcagc ggg [gRNA]	40.00 %	68.84 °C	-	BclI	1 [detail]	20 [detail]	995 [detail]

Showing 1 to 20 of 308 entries

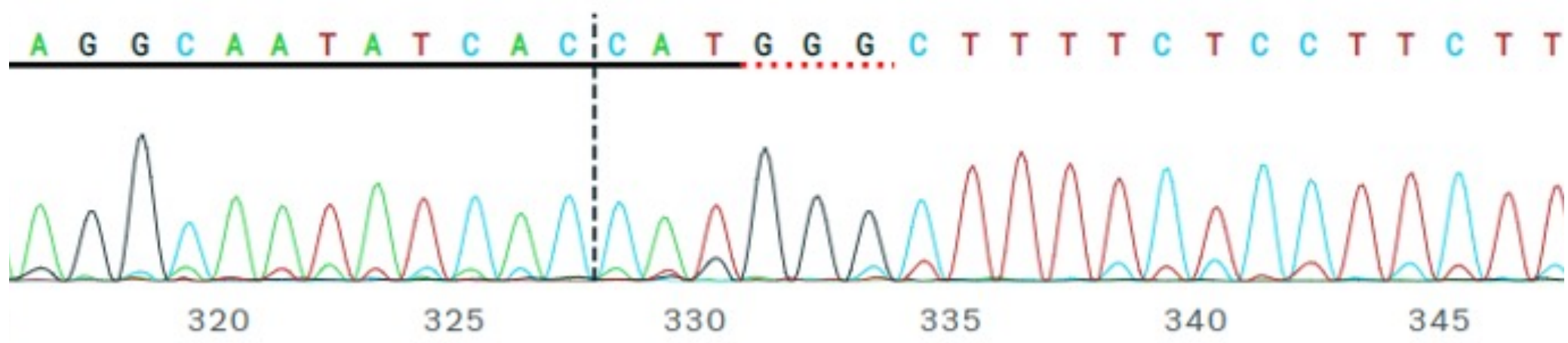
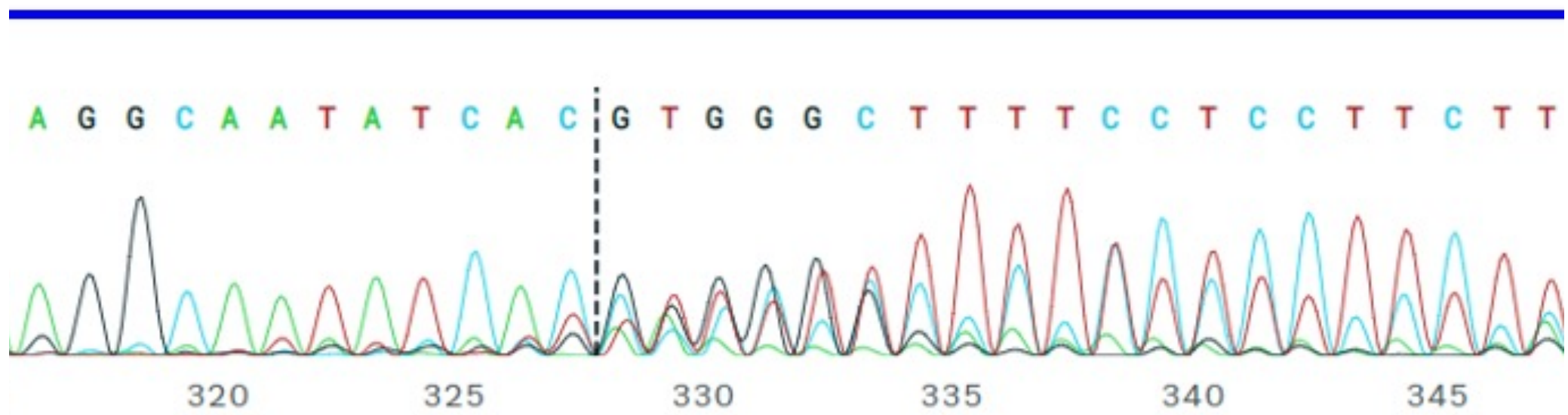
ICE CRISPR Analysis Tool — analyze CRISPR editing results

upload Sanger sequencing data and specify a guide sequence(s) to assess the



This is the Sanger sequence view showing edited and wild-type (control) sequences in the region around the guide sequence. This shows sequence base calls from both the control and the experimental sample .ab1 files, which will contain mixed base calls. The horizontal black underlined region represents the guide sequence. The horizontal red underline is the PAM site. The vertical black dotted line represents the actual cut site. Cutting and error-prone repair usually results in mixed sequencing bases after the cut.



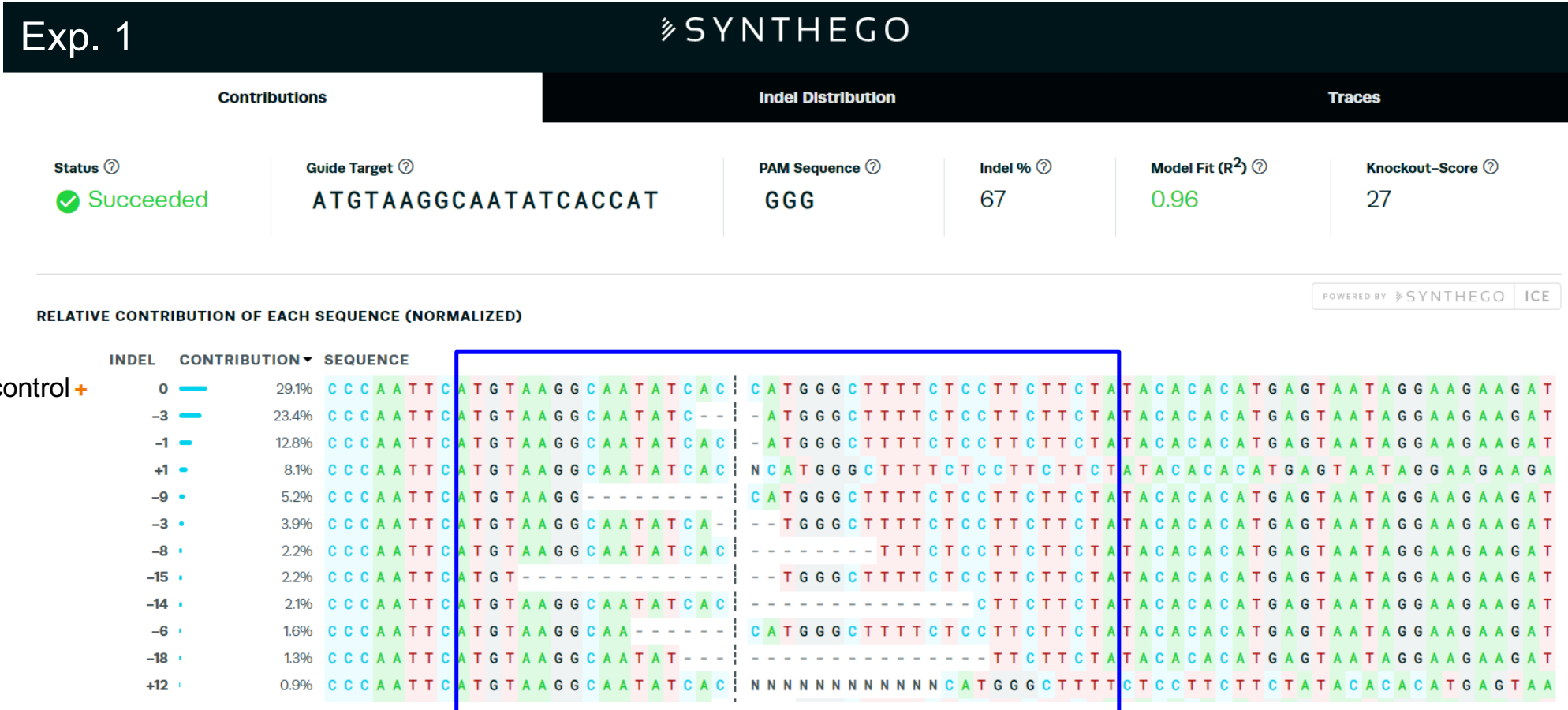


ICE CRISPR Analysis Tool — analyze CRISPR editing results

upload Sanger sequencing data and specify a guide sequence(s) to assess the edits



Result in different indel mutation patterns



Articles in the [April 20, 2016 Issue of Crop Biotech Update](#)

USDA DECLARES GENE-EDITED MUSHROOMS AS UNREGULATED

The U.S. Department of Agriculture (USDA) will not regulate the [genetically modified white button mushrooms developed using CRISPR-Cas9](#). According to USDA, the Animal and Plant Health Inspection Service (APHIS) does not consider the CRISPR/Cas9-edited white button mushrooms to be regulated because it does not contain foreign DNA from plant pests such as viruses or bacteria.

The CRISPR/Cas9-edited white button mushrooms can resist browning, thus improving the appearance and shelf life of mushroom, and allowing automated mechanical harvesting. The anti-browning characteristic was expressed by deleting some [genes](#) that code for polyphenol oxidase (PPO), an enzyme that causes browning.



Jose A. Bemat Bacete/Getty Images

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

Get more details from [APHIS](#) and [Nature](#).

基因編輯技術改良

雙孢菇

美國農部通過基改洋菇

Gene edited mushroom:

- **Resist browning**
不易變黑
- **Improve the appearance and shelf life**
維持美好外觀
- **Allow automated mechanical harvesting**
利於機械採收

USDA Will Not Regulate CRISPR-Edited Crops

Restrictions will remain on transgenic plants, which contain artificially inserted genes from other species.

Apr 2, 2018
DIANA KWON

Genome editing allows breeders to introduce new traits more precisely, and at a faster rate.



PIXABAY, ANALOGICUS

The US Department of Agriculture (USDA) will not regulate plants that have genome editing, according to [statement](#) released last week (March 28) by t

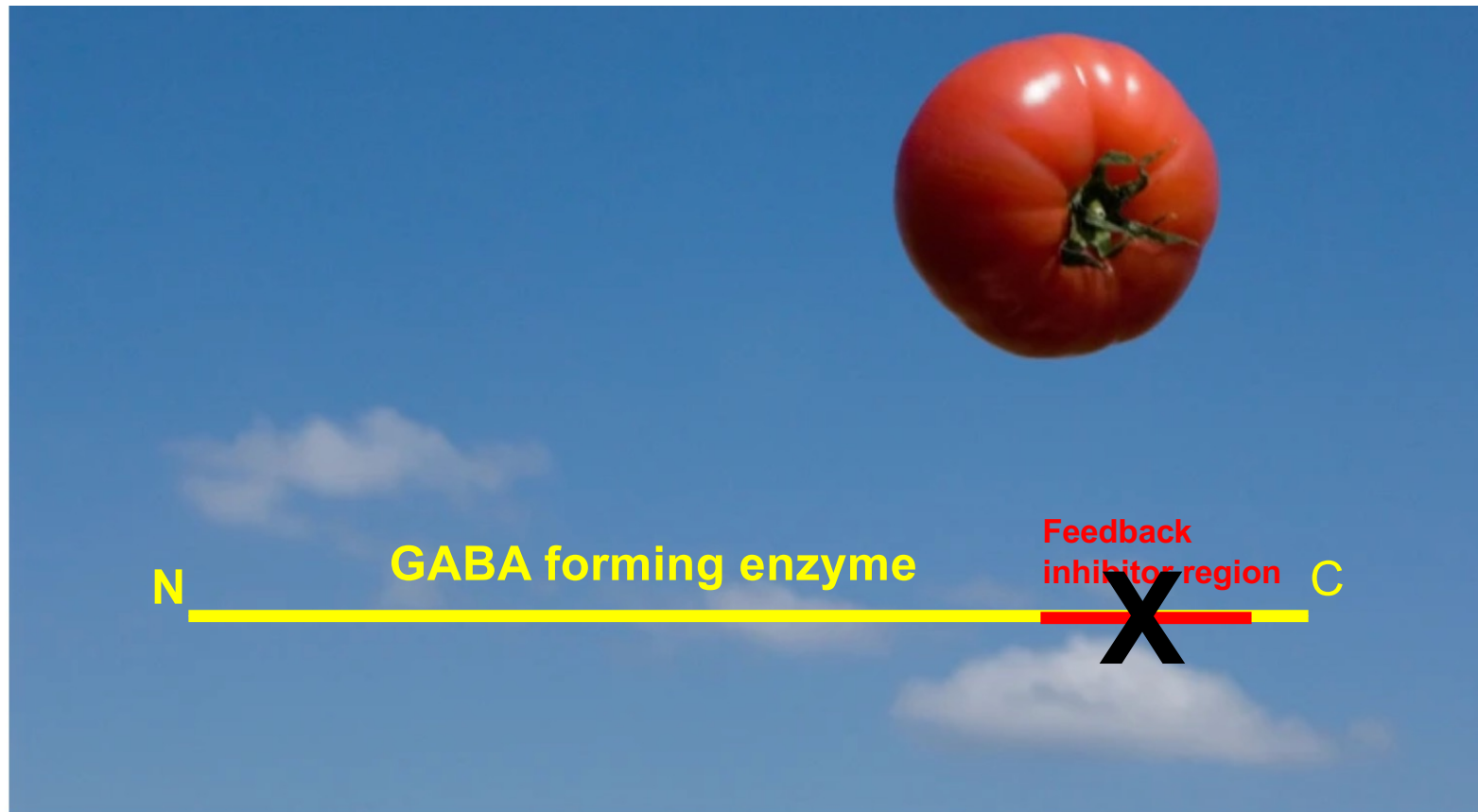
Table 1 CRISPR-edited plants in the pipeline that USDA will not oversee

Date of USDA response	Inquiring institution (location)	Plant trait engineered with CRISPR-Cas9
10/16/2017	USDA ARS, Plant Science Research Unit (St. Paul, Minnesota)	<u>Soybean (<i>Glycine max</i>) with drought and salt tolerance; achieved by disrupting the <i>Drb2a</i> and <i>Drb2b</i> genes (double-stranded RNA-binding protein2 genes)</u>
8/29/2017	Yield10 Bioscience (Woburn, Massachusetts)	Camelina with increased oil content; target genes not disclosed
4/07/2017	Donald Danforth Plant Science Center (St. Louis)	<i>Setaria viridis</i> , or green bristlegrass, with delayed flowering time; achieved by deactivating the <i>S. viridis</i> homolog of the <i>Zea mays</i> ID1 gene
4/18/2016	DuPont Pioneer (Johnston, Iowa)	<u>Waxy corn with starch composed exclusively of amylopectin; achieved by inactivating the endogenous waxy gene <i>Wx1</i> that encodes a granule-bound starch synthase catalyzing production of amylose</u>
4/13/2016	The Pennsylvania State University (University Park, Pennsylvania)	<u>White button mushroom (<i>Agaricus bisporus</i>) with anti-browning properties; achieved by knocking out a gene coding for polyphenol oxidase (<i>PPO</i>)</u>

Source: USDA

GABA-enriched tomato is first CRISPR-edited food to enter market

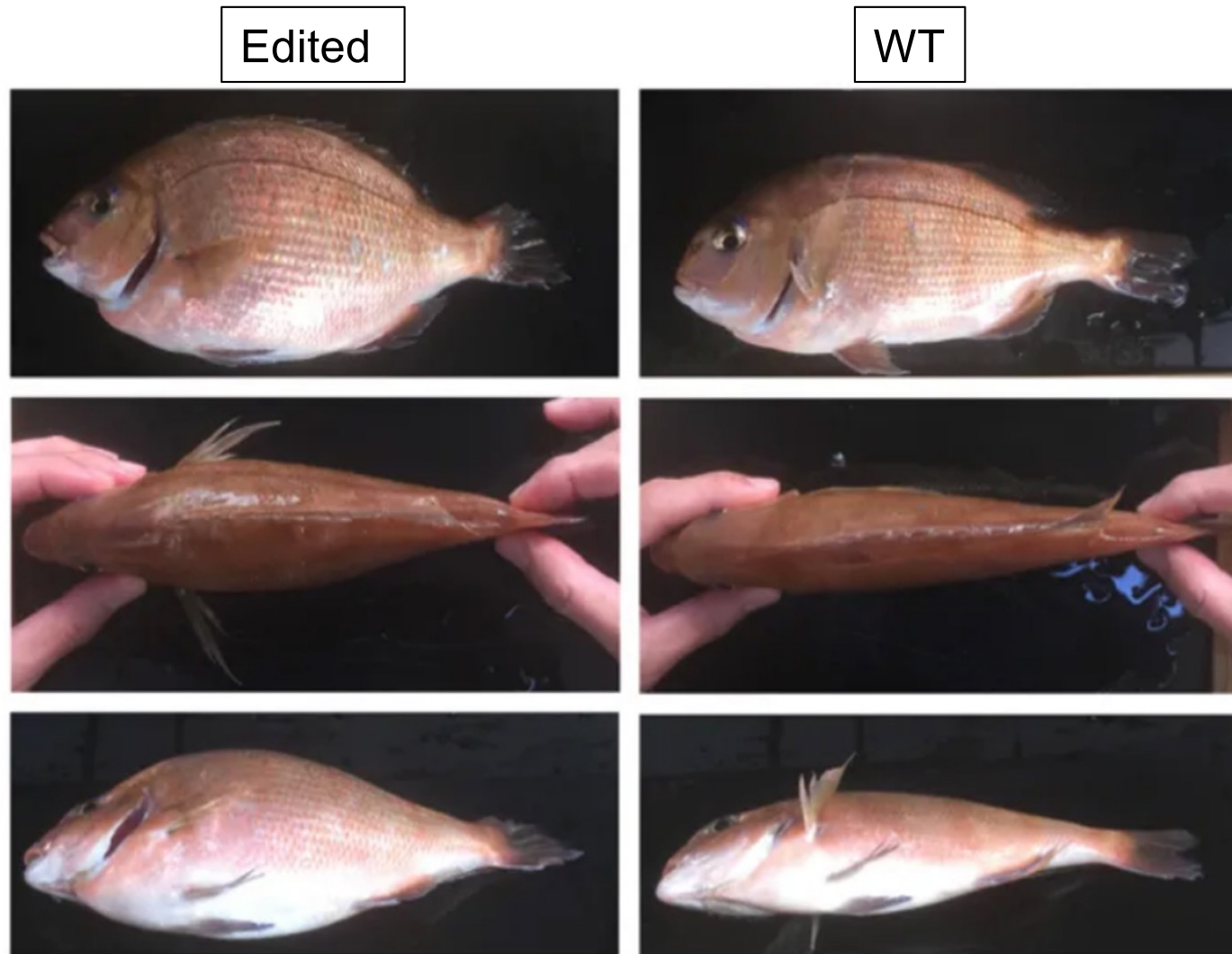
Sanatech Seed's Sicilian Rouge CRISPR-edited 'health-promoting' tomatoes reach consumers and may open the market to more genome-edited fruit, vegetables and even fish.



A CRISPR-edited tomato containing higher GABA than its unedited counterparts takes off in Japan. Credit: Aflo Co., Ltd. / Alamy Stock Photo

Japan Approves Genome-Edited Fishes and Tomato for Commercial Sale

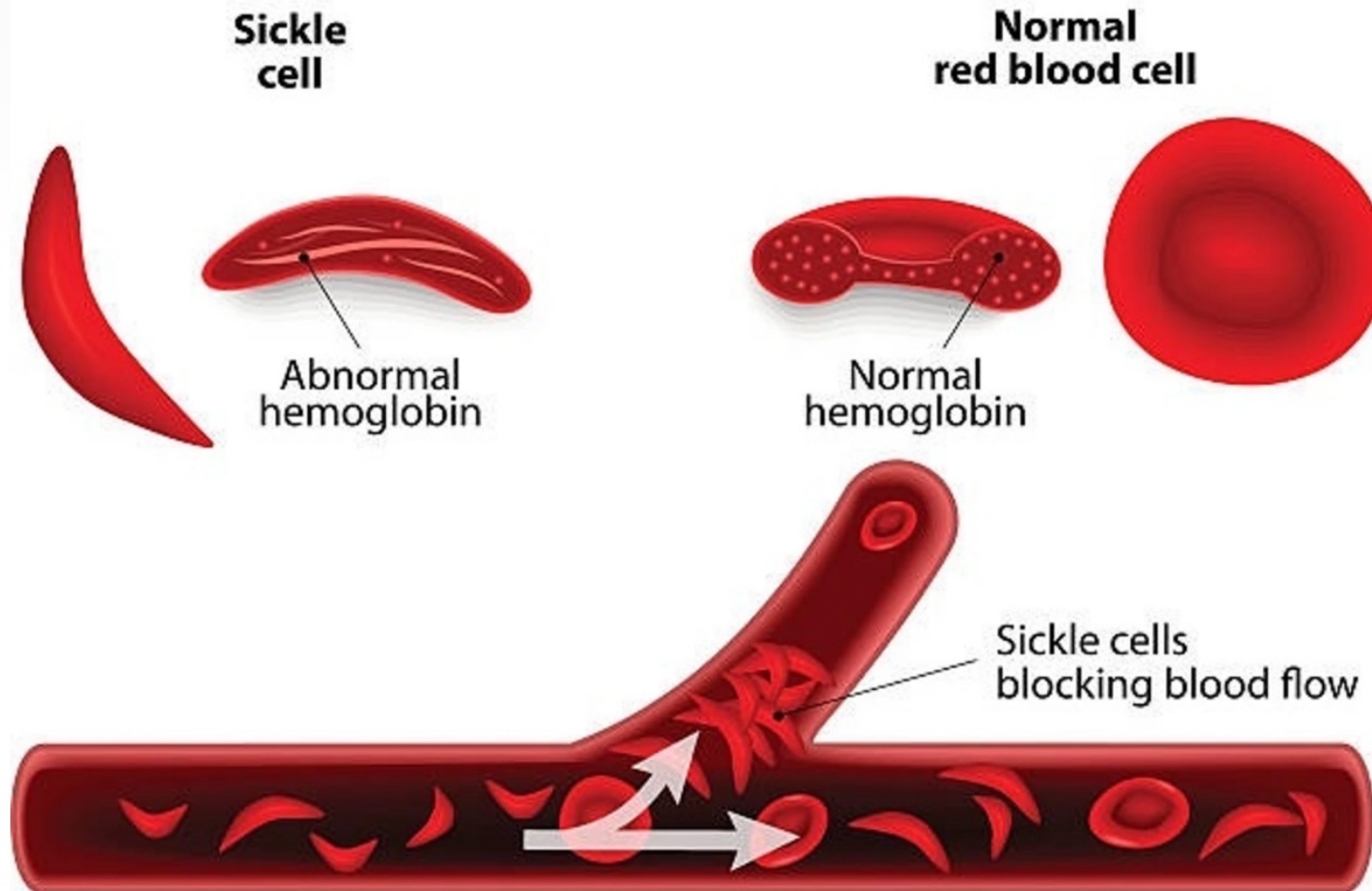
In October and November 2021, two genome-edited fishes were approved for commercial sale in Japan.



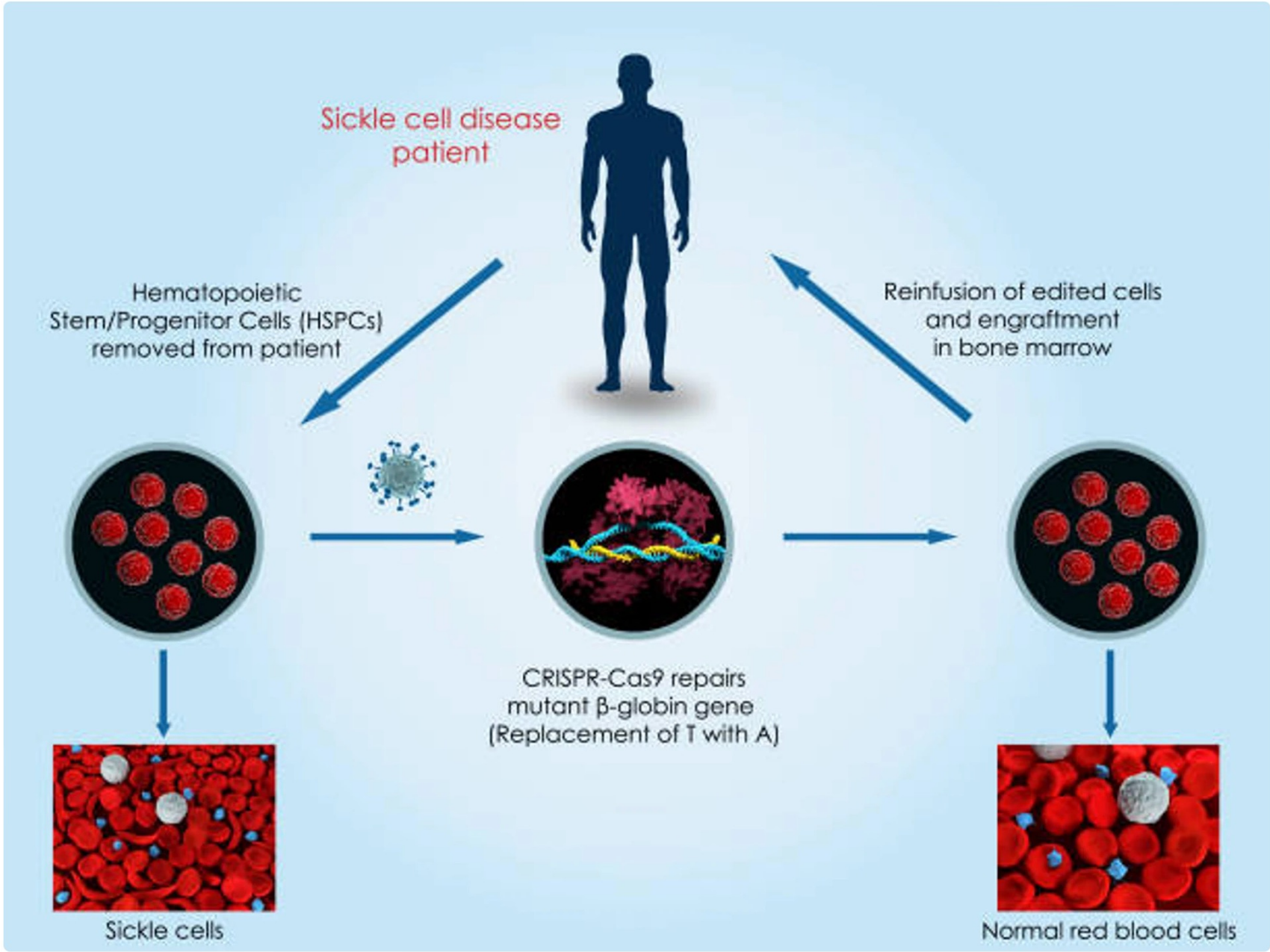
The gene-edited red sea bream (left) compared to unedited versions (right) Photo Source: Dr. Masato Kinoshita, Kyoto University and Dr. Keitaro Kato, Kindai University

FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease

The hemoglobin gene sickle cell anemia (鎌状細胞性貧血) has a single amino acid mutation



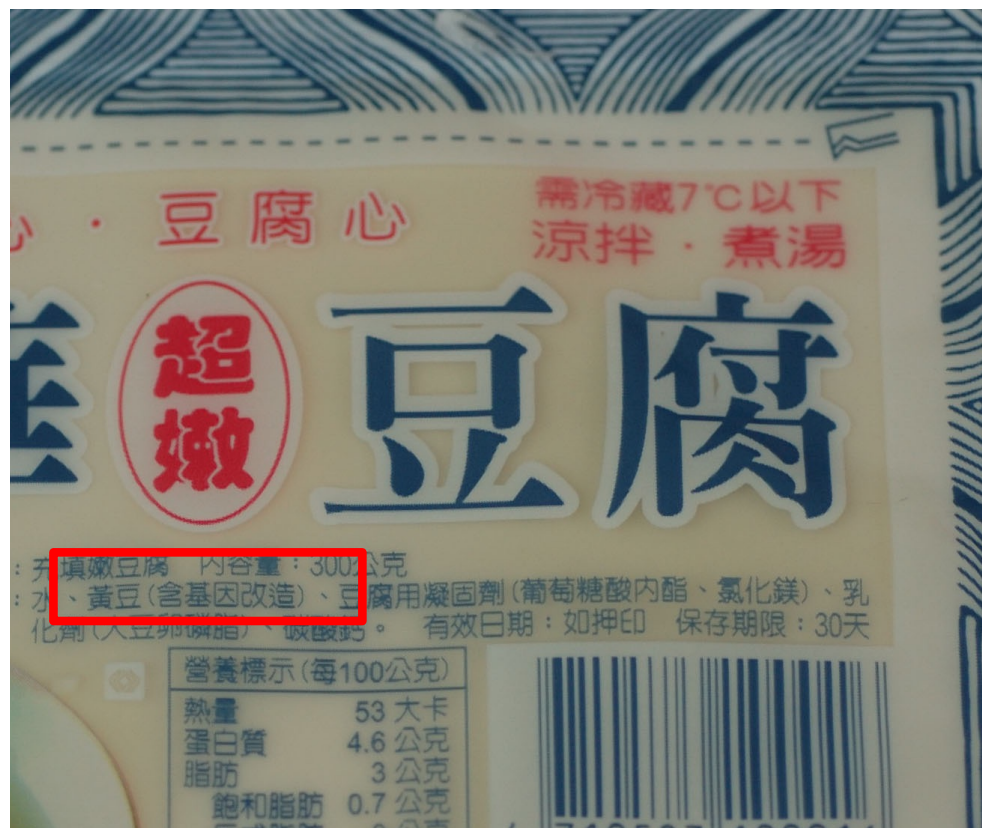
Approaches to CRISPR Sickle Cell Disease Gene Therapy



- ★ 台灣使用之大豆都是由美國及巴西進口, 85%為基改大豆。
市場中到處可見基改豆製品。
- ★ 非法基改木瓜也到處可見。

但是國內研發之基改動植物, 如螢光魚, 植酸米等,
卻無法得到合理的認證。

- ★ 台灣目前沒有基因編輯的政策。



謝謝 您對基因工程及基因編輯
有關議題的關注與興趣 !!

Q&A, Q&A and more Q&A! ! !