Advancing Frontiers of Biotechnology:
Exploring Gene Editing in Crops and Animals

GENE EDITING IN CROPS

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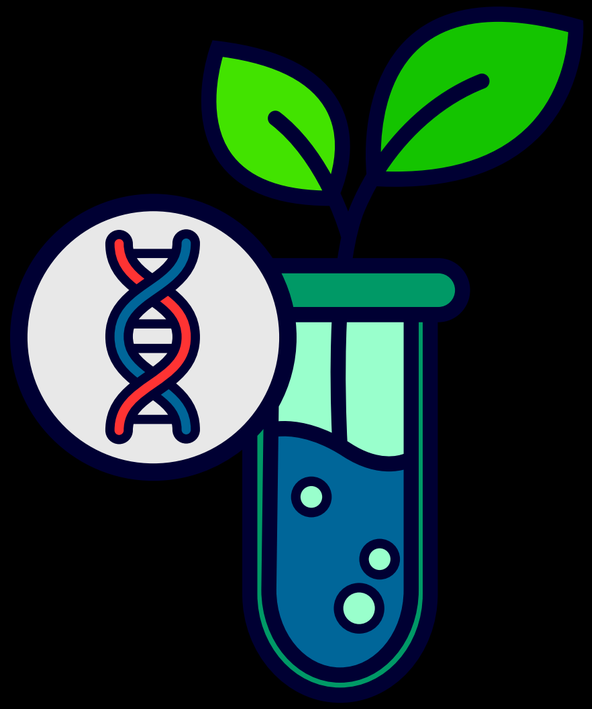
LOS BAÑOS



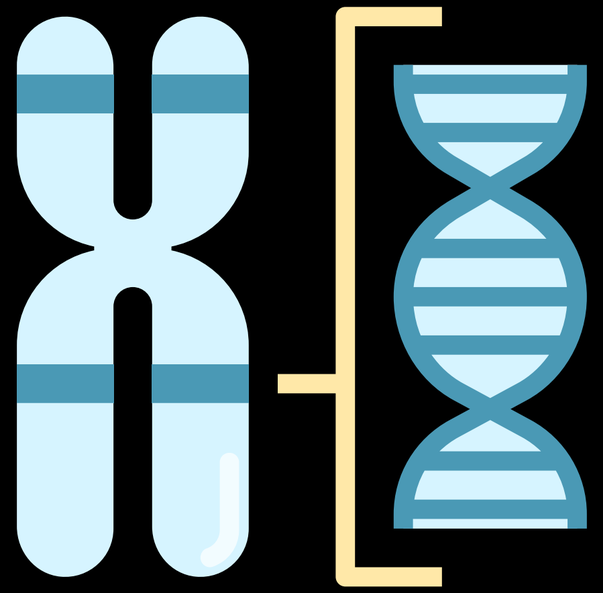
PRESENTATION OUTLINE

GENE EDITING DEFINITION

CRISPR CAS 9 SYSTEM

GMO VS GED

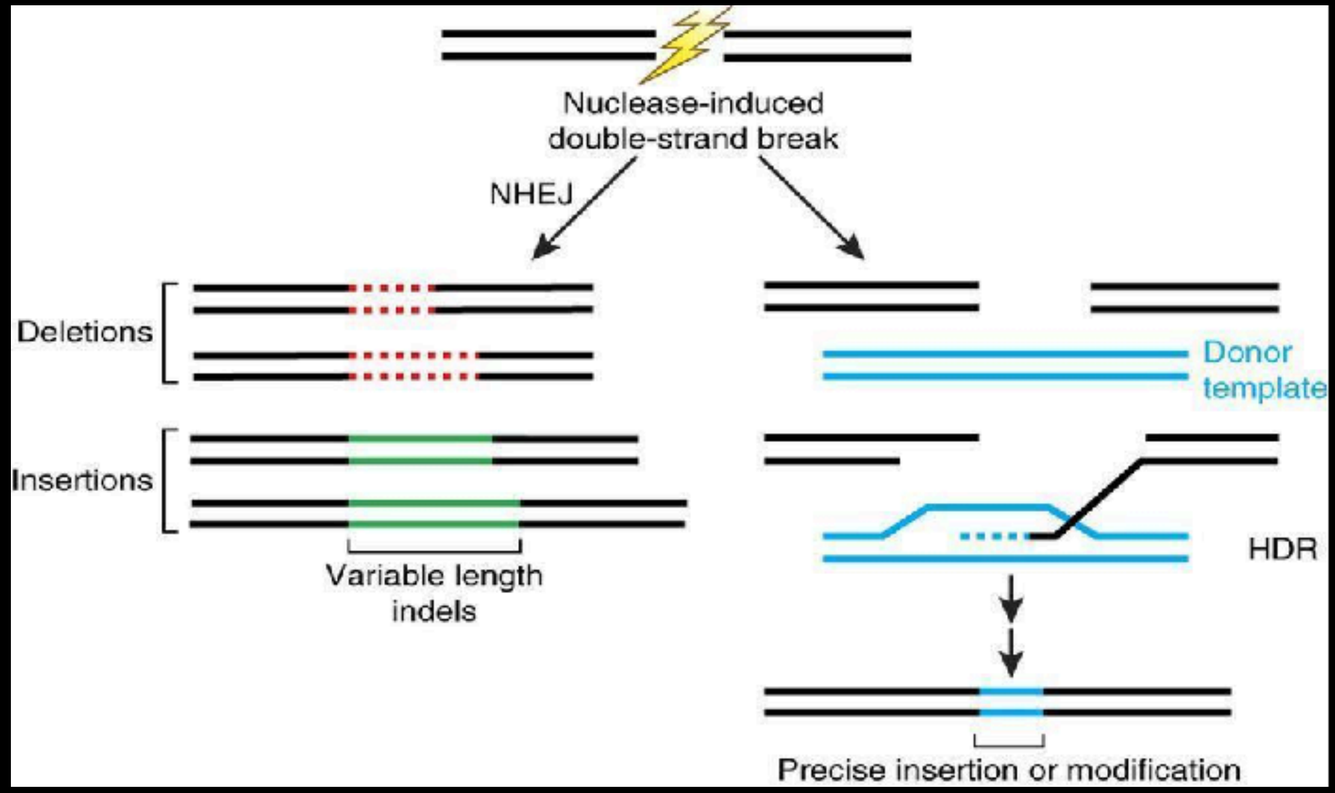
PRODUCTS OF GENE EDITING



GENE/GENOME EDITING

Genetic mutation (gene disruption, gene insertion) of a pre-determined genetic locus/ region in a genome using site specific nucleases.

site directed mutagenesis



Sander and Jung 2014

EDITING

1. The girl is smart.
2. That girl is smart.
3. She is smart.
4. Anna is smart.
5. She is intelligent.
6. The girl is beautiful and smart.
7. The beautiful girl is smart.

Non-homologous end joining (NHEJ)

Homology-directed repair (HDR)

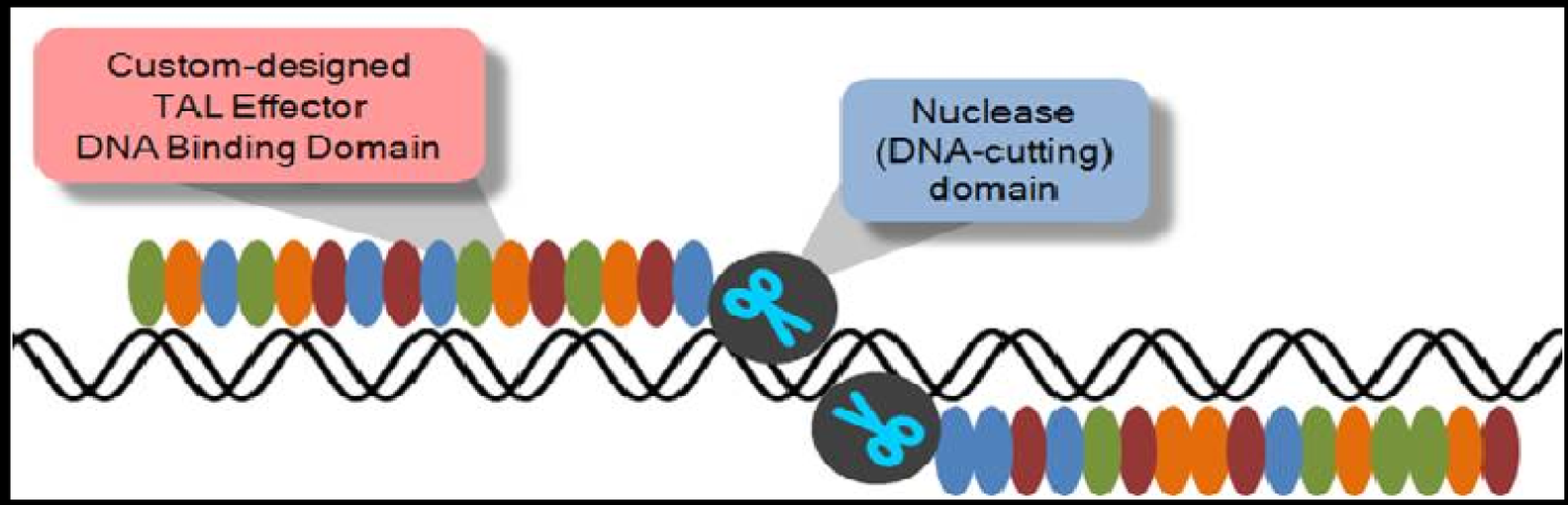
1st GEN GMO

Site-specific endonucleases (SSNs)

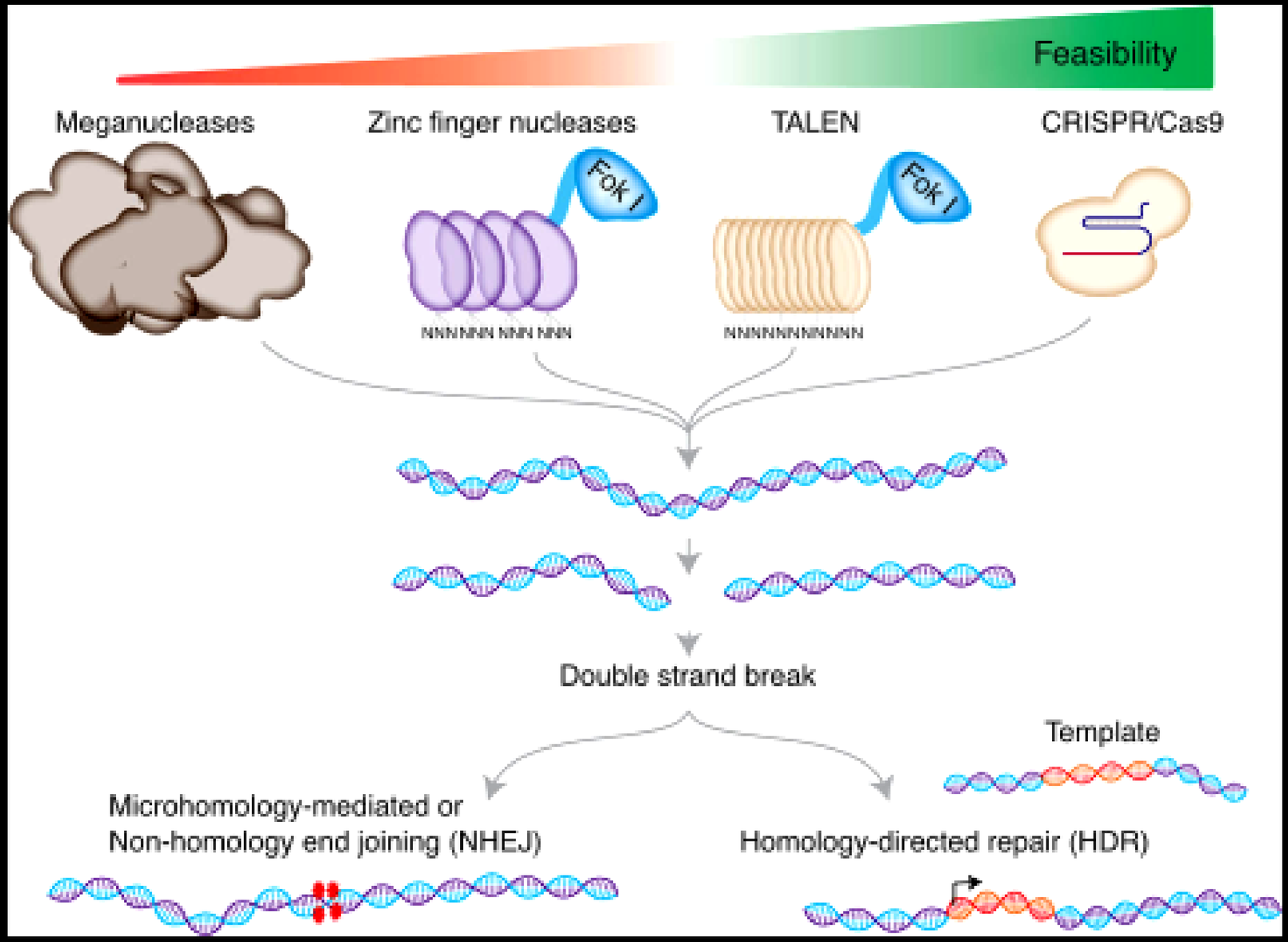
recognize unique sequences and specifically cleave DNA at unique sites within the genome;

“magic scissors”

Fusion of a binding domain and nuclease domain



Morbitzer et al., 2011

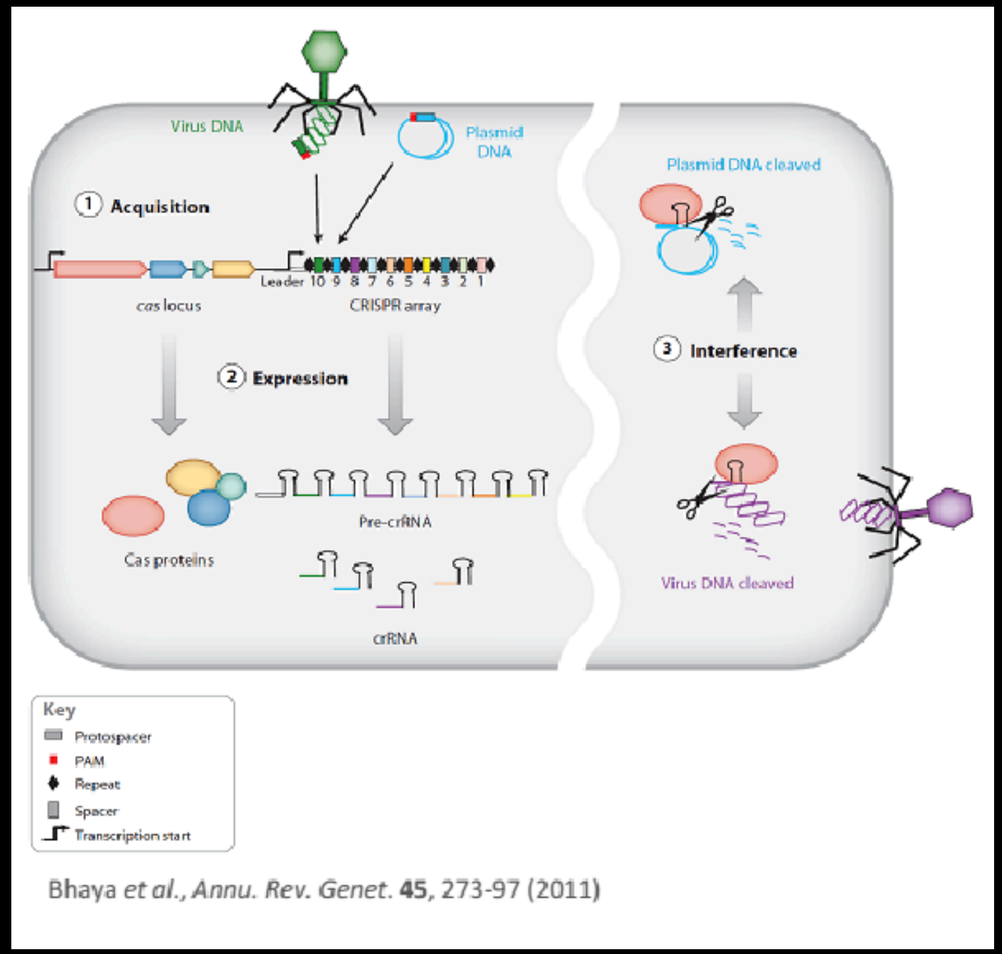


Mazhar Adli - review article : The CRISPR tool kit for genome editing and beyond

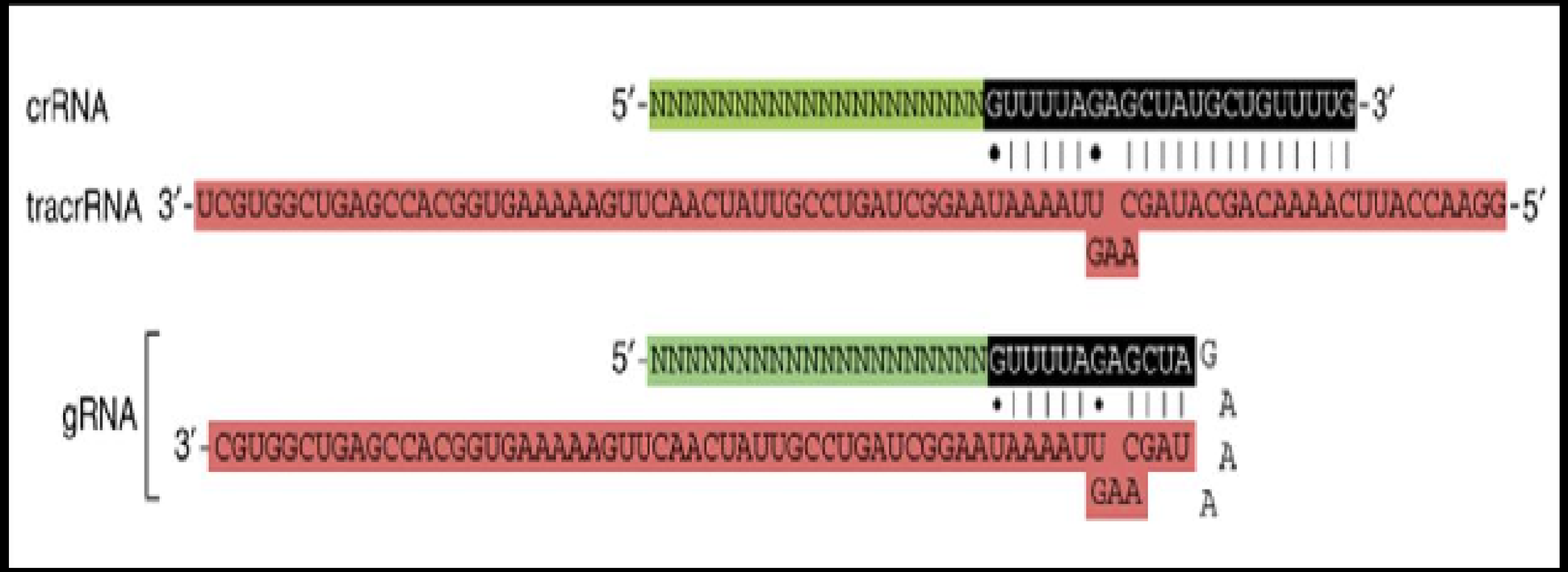
DOI: 10.1038/s41467-018-04252-2

CRISPR-Cas 9 system

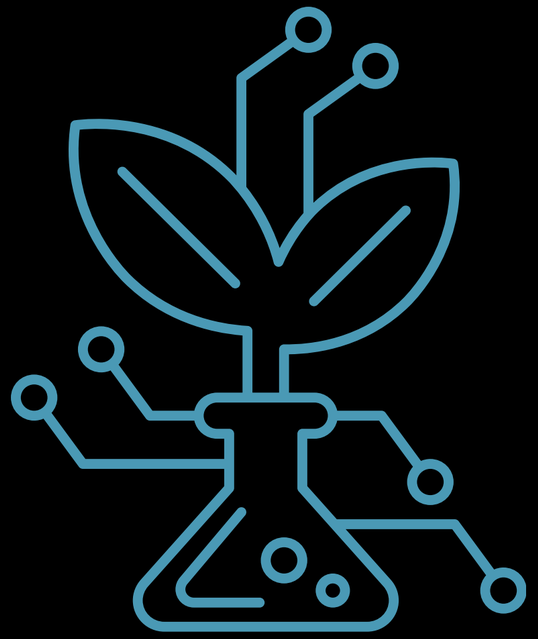
The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material



Bhaya *et al.*, *Annu. Rev. Genet.* **45**, 273-97 (2011)



reliant on the design of the guide RNA rather than the engineering of the protein itself. This creates an advantage as several RNA guides can be designed to edit the target gene at a faster rate and at a more affordable cost (Boglioli and Richard, 2015).



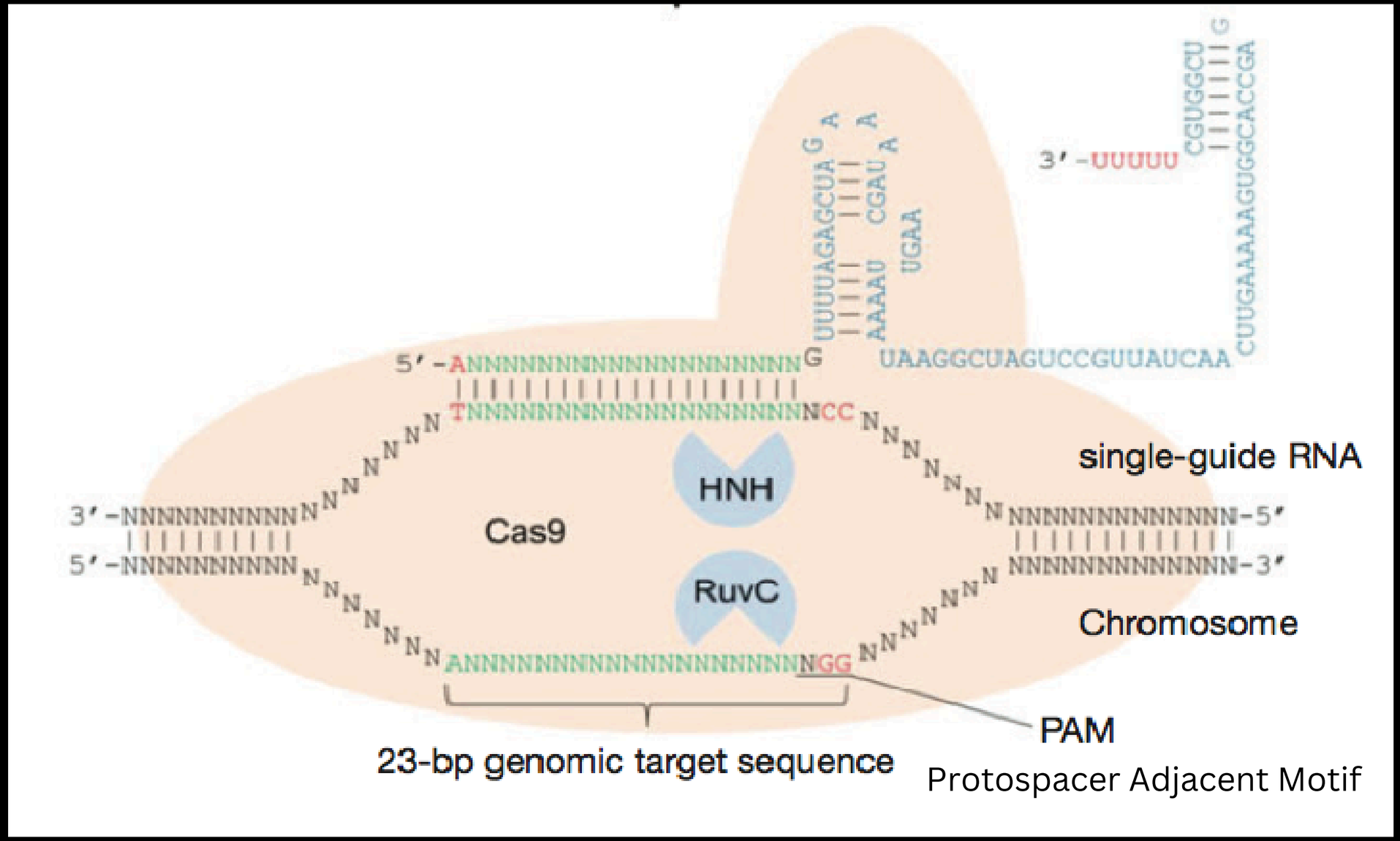
GENE EDITING REQUIREMENTS

GENOME INFORMATION

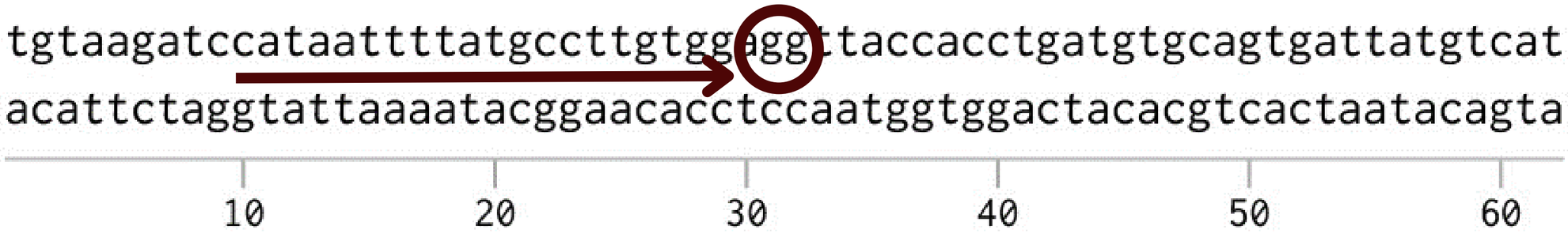
TRANSFORMATION PROCESS

REGENERATION PROTOCOL

ASSAY SYSTEM



Shan et al, 2013


tgtaagatccataattttatgccttgtggaggttaccacctgatgtgcagtgattatgtcat
acattctaggtattaaaatacggaacacctccaatggtggactacacgtcactaatacagta
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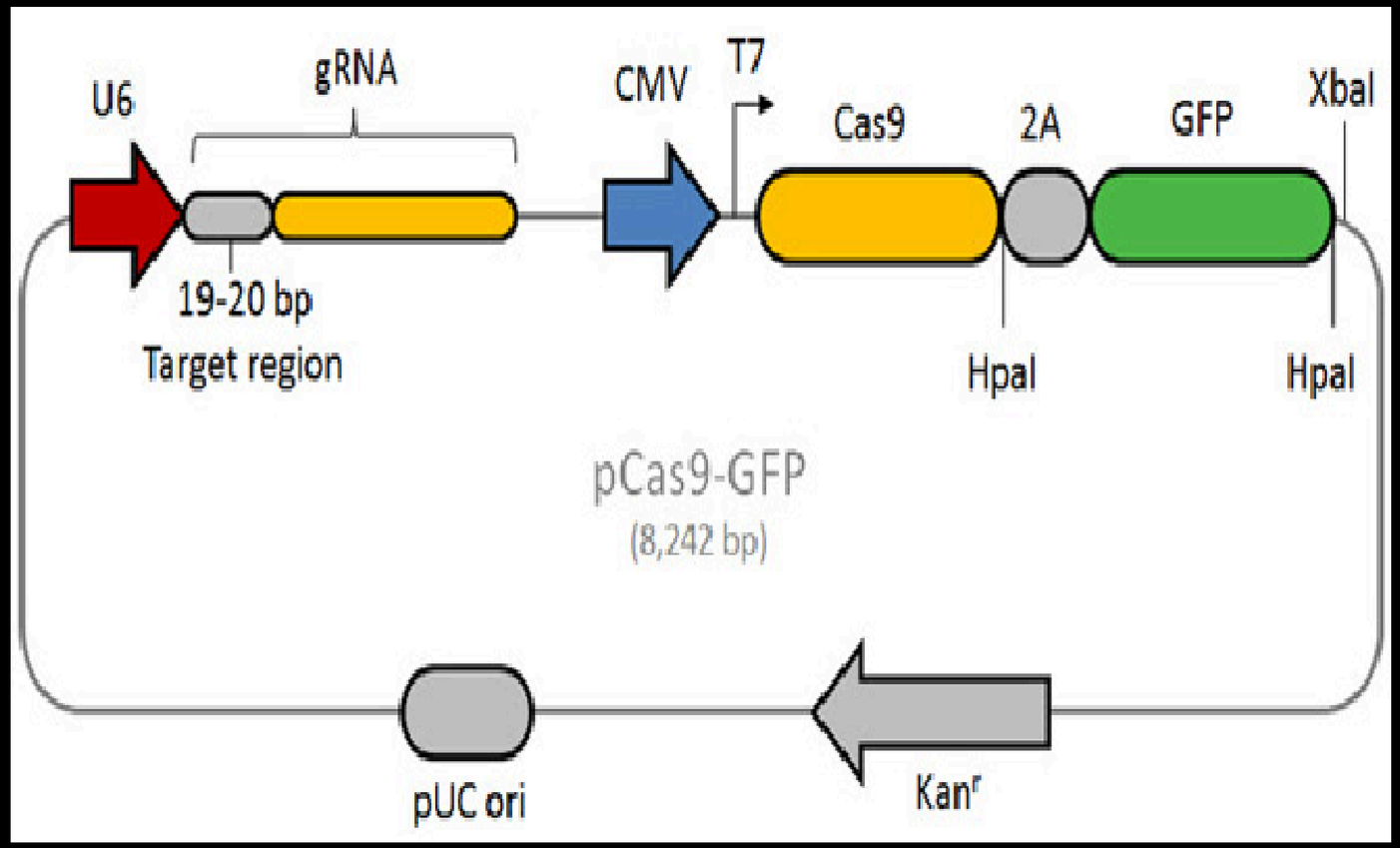
Free gRNA design software

- https://www.synthego.com/products/bioinformatics/crispr-design-tool
- https://www.benchling.com/crispr/
- https://horizondiscovery.com/en/products/tools/CRISPR-Design-Tool
- https://chopchop.cbu.uib.no/
- https://www.atum.bio/eCommerce/cas9/input
- https://www.genscript.com/gRNA-design-tool.html

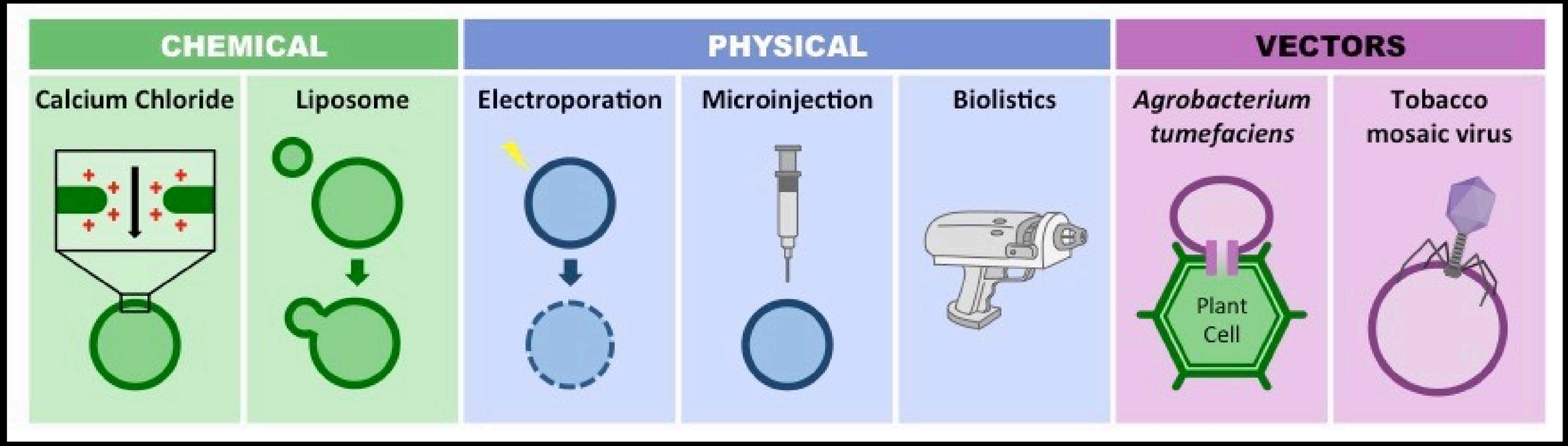
CRISPR-Cas 9 Binary Vector



https://www.sigmaaldrich.com/content/dam/sigma-aldrich/articles/biology/Cas9-figure1.jpg

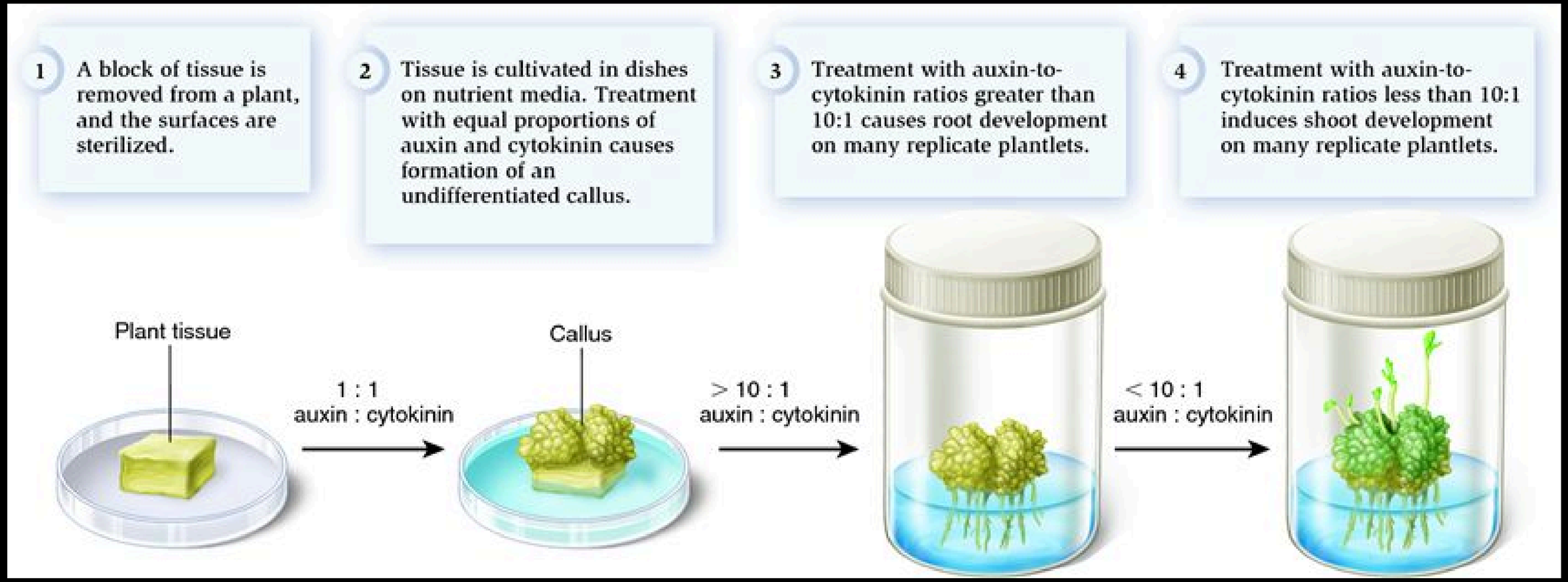
Promoters: 35S CaMV, U6
Terminator: Nos
Plant selectable marker gene: NPTII or Kan resistance gene
GOI: gRNA, Cas9-GFP
Transfer: RB and LB

Transformation/ transfection techniques

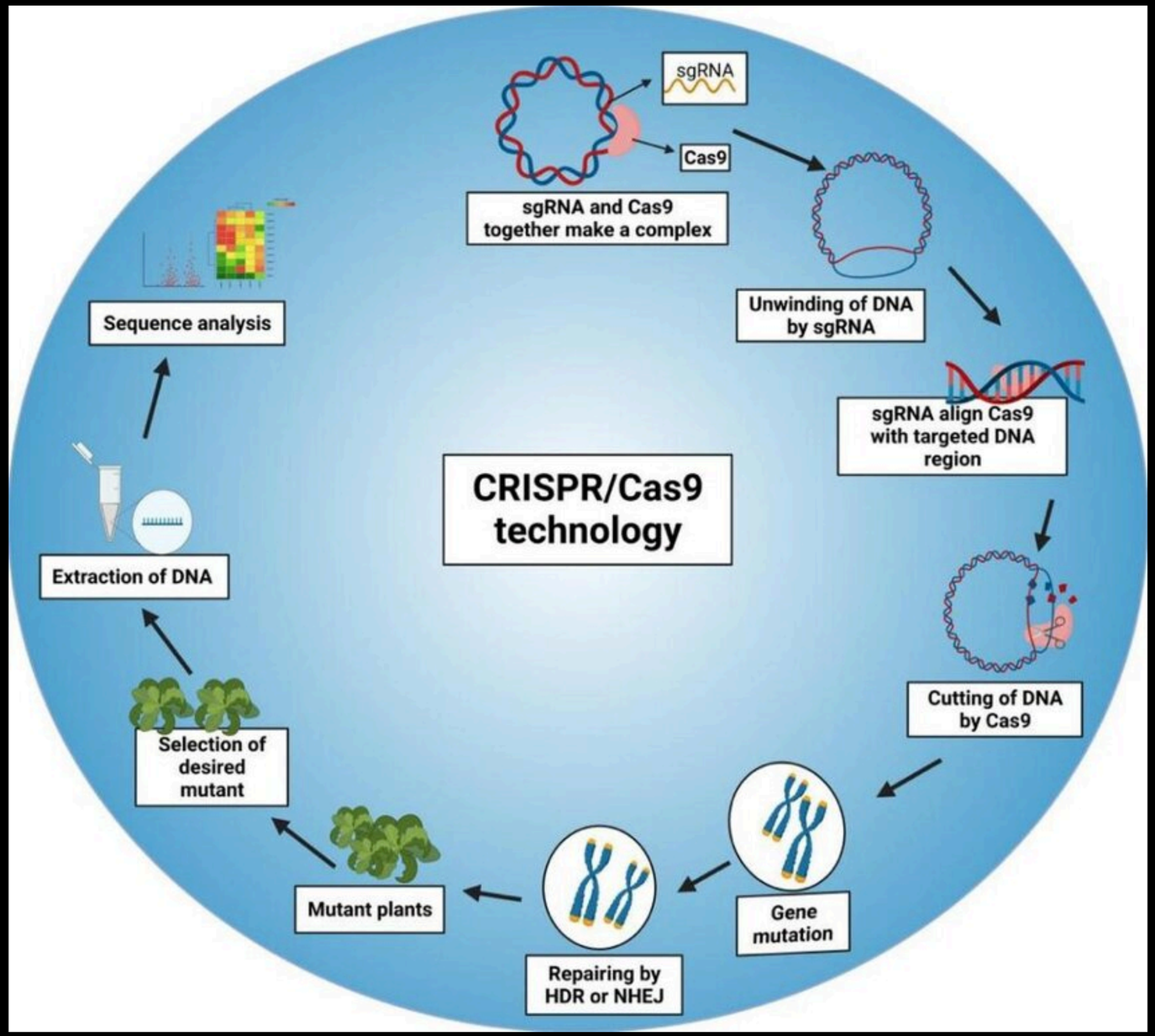


https://ib.bioninja.com.au/_Media/gene-delivery-systems_med.jpeg

Plant tissue culture is very critical in genome editing of plants

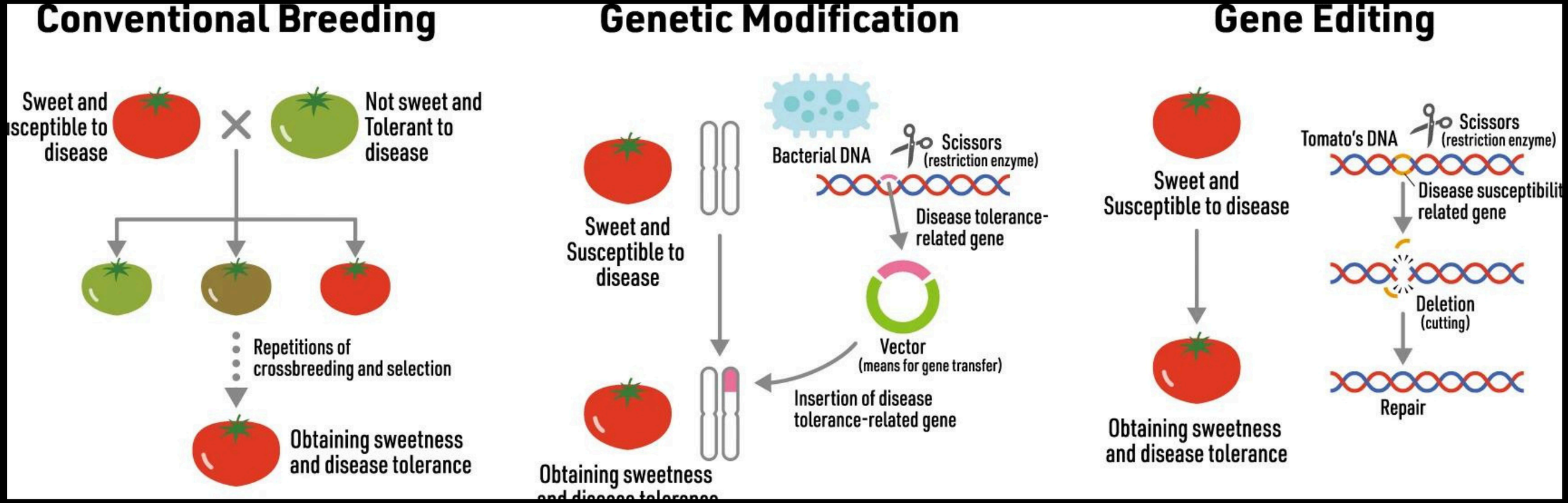


http://tissueculture ofplants.blogspot.com/p/tissue-culture.html



Rasheed et al, 2022

DOI:10.1007/s11033-022-07529

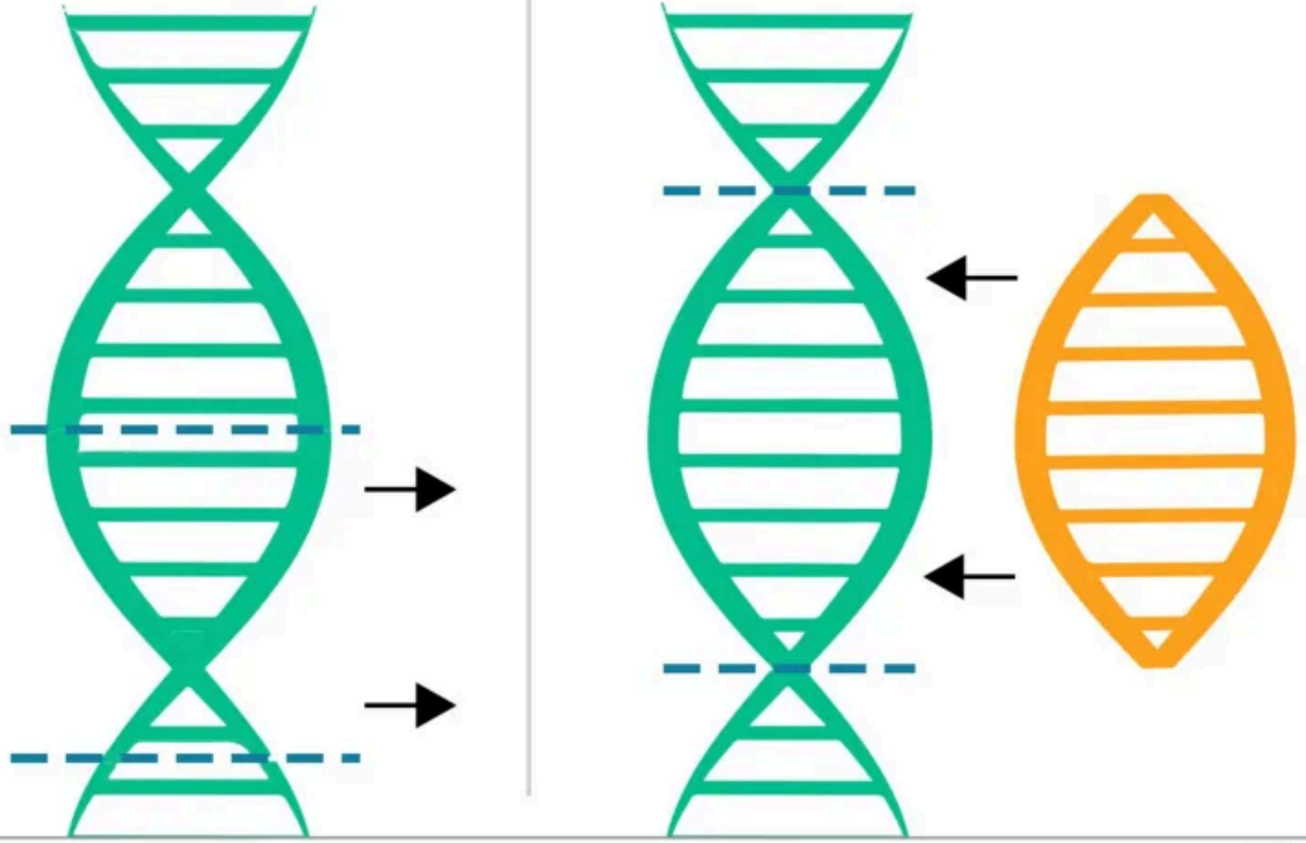


Kato-Nitta et al, 2019

The difference between gene editing and modification

Gene editing
Section of the plant's DNA snipped out

Gene modification
Section of DNA added, sometimes from a different species



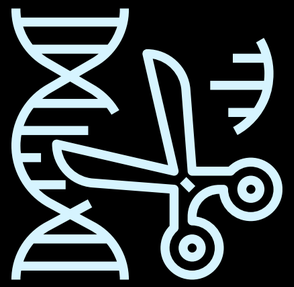
Source: BBC research

BBC

Gene Edited Mushrooms

U.S. Department of Agriculture/Flickr, CC BY 2.0

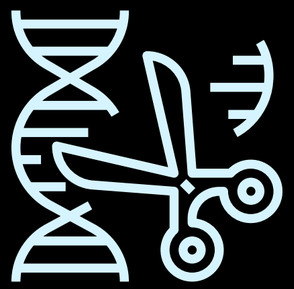
- Yinong Yang in Penn State Univ
- mushrooms are a big cash crop in Pennsylvania
- knock-out of one of six polyphenol oxidase genes (PPO)
- reduction of activity by 30%
- first organism engineered by CRISPR–Cas9 to get a green-light from the U.S. government
- no detection of foreign DNA from virus or bacteria



Gene Edited Soybean

From calyxt.com

- This is the first gene-edited food released for consumers in the US
- 80% higher in oleic acid, 20% less in saturated fatty acids, has 0 grams trans fat per serving, has three times the fry-life and a longer shelf-life
- developed using TALENs by introducing stacked mutations in two fatty acid desaturase 2 genes (FAD2-1A and FAD2-1B)



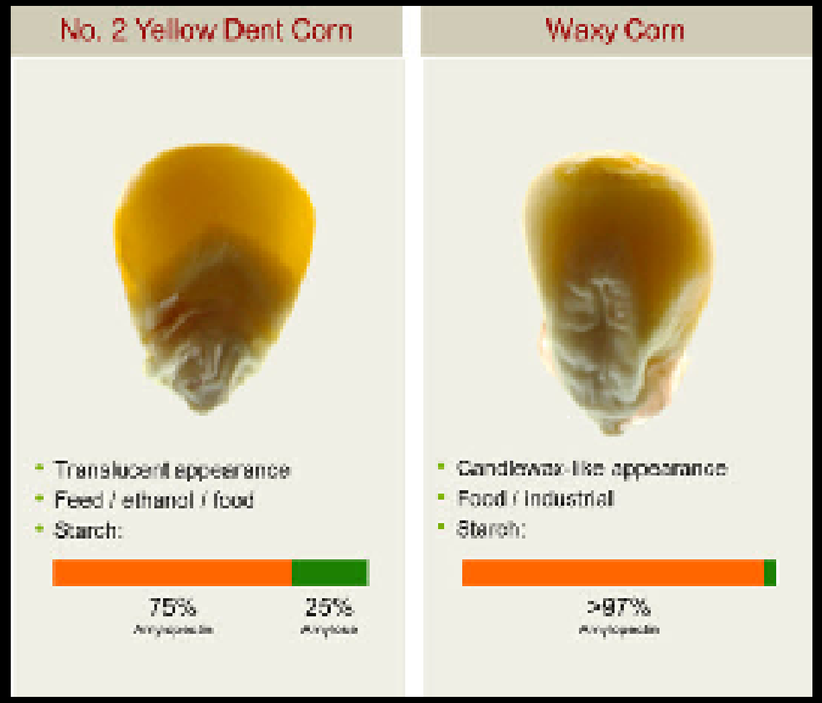


Photo Credit: Dow Dupont

Gene edited Waxy corn

- developed by Dow Dupont Pioneer/ Corteva
- all amylopectin and this specialty starch is used in industry
- waxy gene has been known to science for almost 100 years and used for conventional breeding
- fourth genome-edited food product that Japan did not subject to regulations for genetically engineered (GE) food

First Gene Edited Product Approved in the Philippines

https://tropic.bio/wp-content/uploads/2023/04/Tropic-green-bananas-1536x928.jpg

- Tropic's Non Browning Gene edited Banana
- Gene editing inducing Gene Silencing (GEiGS)
- Non-GMO exemption
- importation and propagation

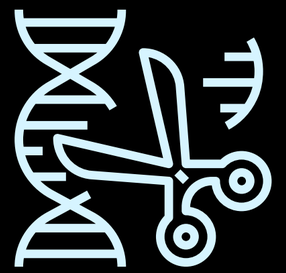
Photo Credit: Sanatech Seed

GABA enriched Tomato

- developed by Hiroshi Ezura at the University of Tsukuba in Japan
- 5 times the amount of Gamma Aminobutyric Acid
- now commercialized in Japan
- High GABA tomato is the second gene-edited crop that has been determined as non-GMO in the Philippines

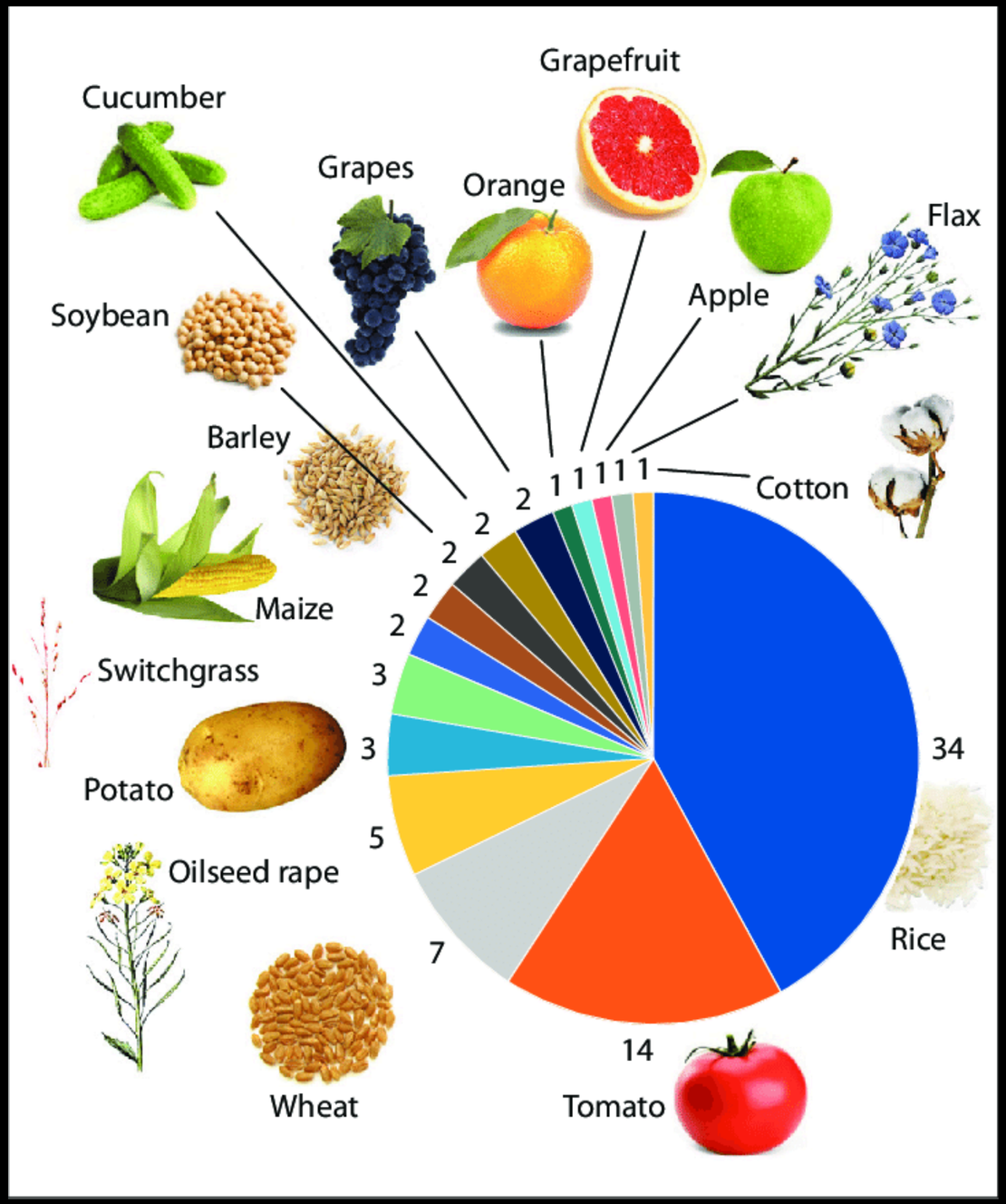
Crop	Trait	Edited genes	Stage
 Banana	Disease resistance (BXW, Fusarium wilt, BSV)	*DMR6*, BSV sequences	3,1
 Cassava	Disease resistance (BB)	*SWEET* gene promoters	3
	Food safety (cyanide-free)	Linamarin synthase	3
	Quality (waxy starch)	*GBSS1*	3
 Maize	Disease resistance (MLN)	*C6 QTL*	1
	Weed resistance (*Striga*)	Strigolactone	3
 Potato	Disease resistance (PVY[a], late blight)	*eIF-4E, StDMR6-1, StCHL1*	2
 Rice	Disease resistance (BLB, RHB)	*SWEET* gene promoters, *AGO4, STV11*	4,3
	Food safety (low arsenic and cadmium)	*OsNRAMP5, OsPT8, LS1, LS2*	3
	Nitrogen remobilization, and methane emission reduction	Unpublished	3
	Insect resistance[a] (BPH)	BPH resistance alleles	2
 Wheat	Disease resistance (rusts, mildew)[a]	*Lr67* and others	3

Pixley et al, 2022
https://doi.org/10.1038
/s41588-022-01046-



Number of genes modified using CRISPR/Cas system with the aim of crops improvement, summarized from (Korotkova et al., 2017) and the Table for the period from August 2013 till August 2018 (Korotkova et al, 2016)

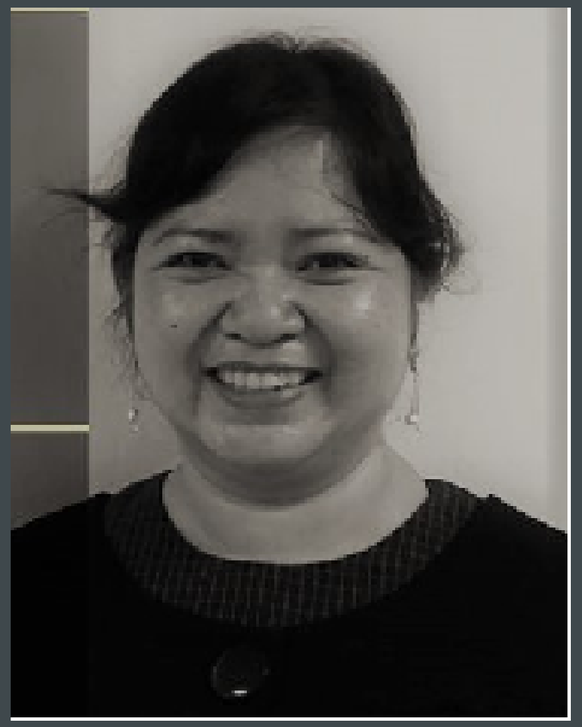
81 genes in 16 crops in 5 years



Korotkova et al., 2017
DOI:10.18699/VJ19.458

TAKE HOME

- Genome editing is targeted mutagenesis
- CRISPR Cas system is the most popular due to its versatility and affordability.
- CRISPR-Cas 9 is a naturally occurring defense mechanism of bacteria against invading virus.
- Gene editing is a promising tool to increase DNA variation, thus giving increased opportunity to develop advances in crops, animal breeding and medicine



Mahalo nui loa

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Any questions?