



**TAL
TECH**

PLANT GENOME EDITING AND THE GREEN DEAL

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GENOME EDITING

To **edit** the genome means to introduce changes into the genome in a **targeted** way. This means, to delete, insert or mutate a DNA sequence in a **precise** manner.

Tools: NGTs (new genomic techniques), in the case of plants those are also called NBTs (New Breeding Techniques). Created since 2001.

- **Main tool:** Site-directed nucleases (SDN) and among them the ones that recognize a specific DNA sequence by complementary RNA molecule (CRISPR/Cas) and not by a protein domain (ZFN, TALEN).



GREEN DEAL

The EU Green Deal is a coordinated set of policies and legislation designed to lower the European Union's global warming emissions to zero over by 2050.

The Green Deal's policies focus on seven areas: **Climate action, Eliminating pollution, Sustainable mobility, Building and renovation, Sustainable Industry, Clean energy, Sustainable agriculture, Biodiversity, From farm to fork** (to ensure more sustainable food systems).



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How Crops are Genetically Modified

Traditional Breeding

Since 1930 Mutagenesis

RNA Interference

Transgenics

Gene Editing

Crossing plants and selecting offspring

Exposing seeds to chemicals or radiation

Switching off selected genes with RNA

Inserting selected genes using recombinant DNA methods

When used to delete genes using engineered nucleases (CRISPR, TALENs, ZFNs, etc.)



Desired gene(s) inserted with other genetic material

Random changes in genome, usually unpredictable

Targeted gene(s) switched off or 'silenced'

Only gene(s) inserted at desired locations selected

Desired gene(s) deleted only at known locations

Almost all crops



Number of genes affected:
few genes to whole genomes

100s - 1,000s

1 - dozens

1 - 8

1 or more

No safety testing required;
Unregulated

No safety testing required;
Unregulated

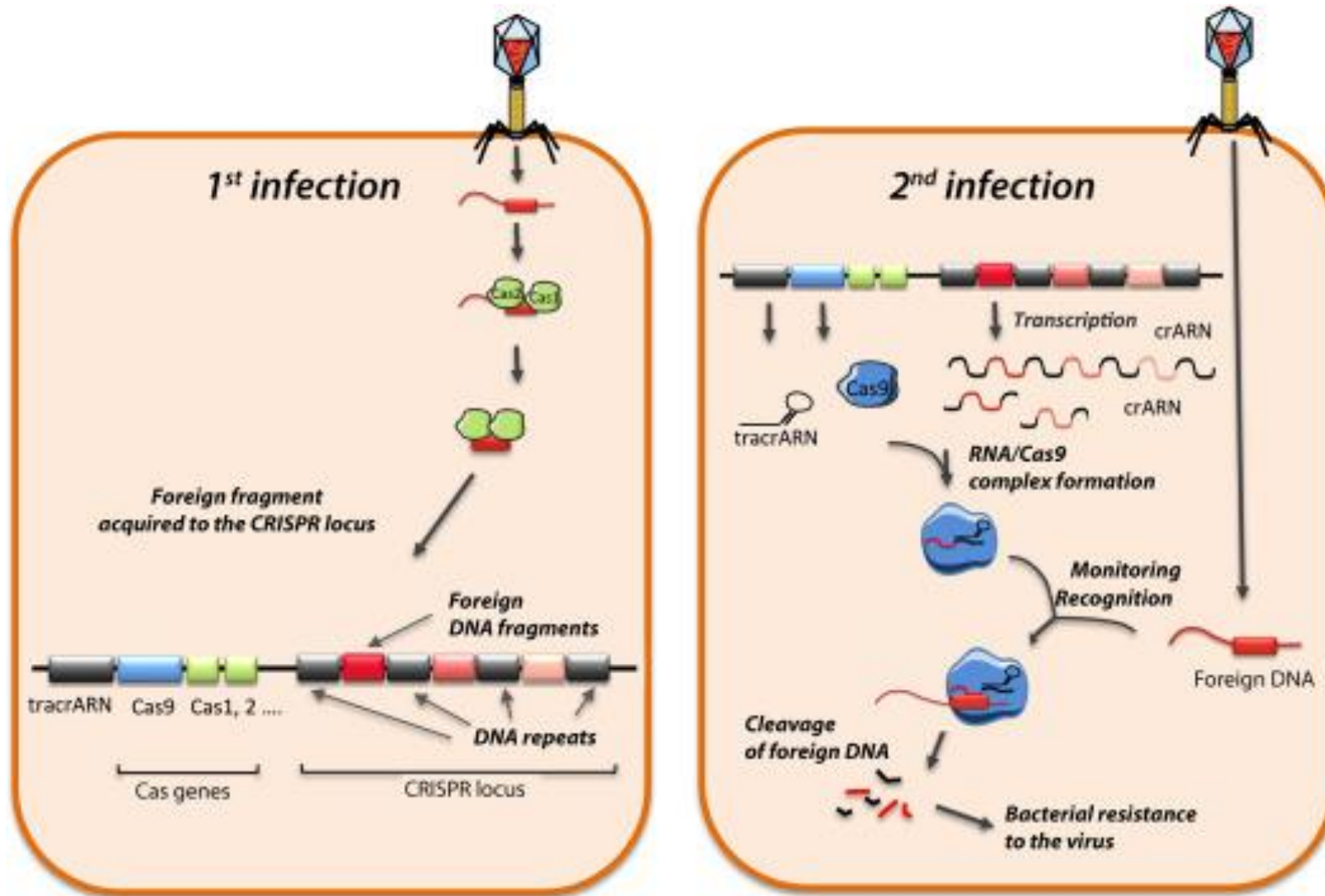
Safety testing required;
Highly regulated

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Highly regulated

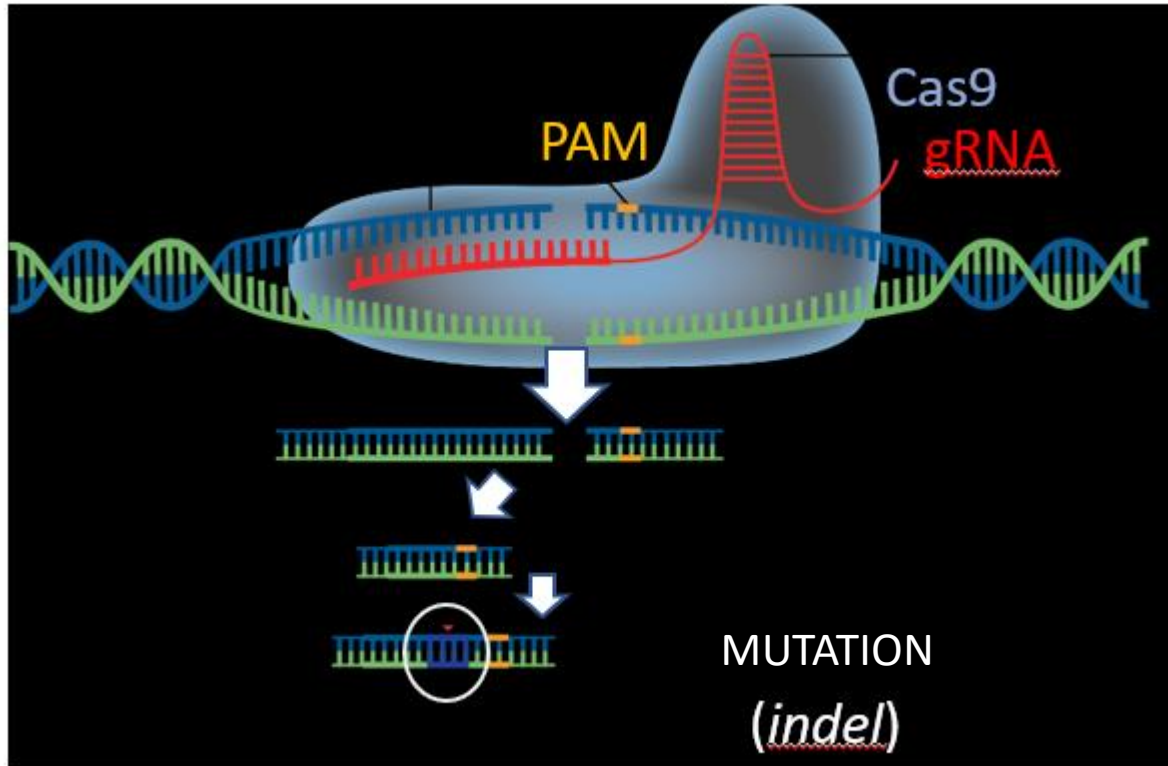
Safety testing required depending on jurisdiction;
Mixed regulations

Undesirable, unintended effects rarely occur in the final product of any crop, regardless which process is used.

CRISPR/Cas, a natural system



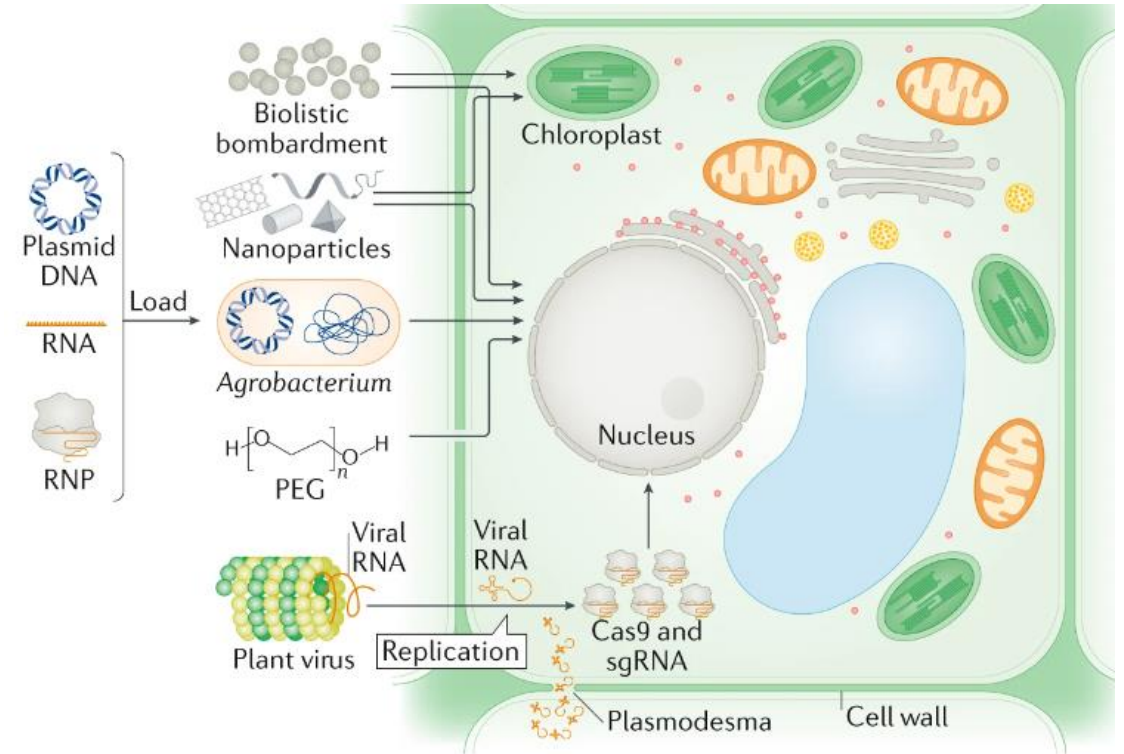
CRISPR/Cas as GE technology



<https://rockland-inc.com/crispr-gene-editing.aspx> (modified)

Protospacer adjacent motifs (PAMs): 2-6 bp.
Cas nucleases recognize PAMs and cannot cleave DNA unless a PAM is present.

gRNA = sgRNA (guide RNA or single guide RNA)

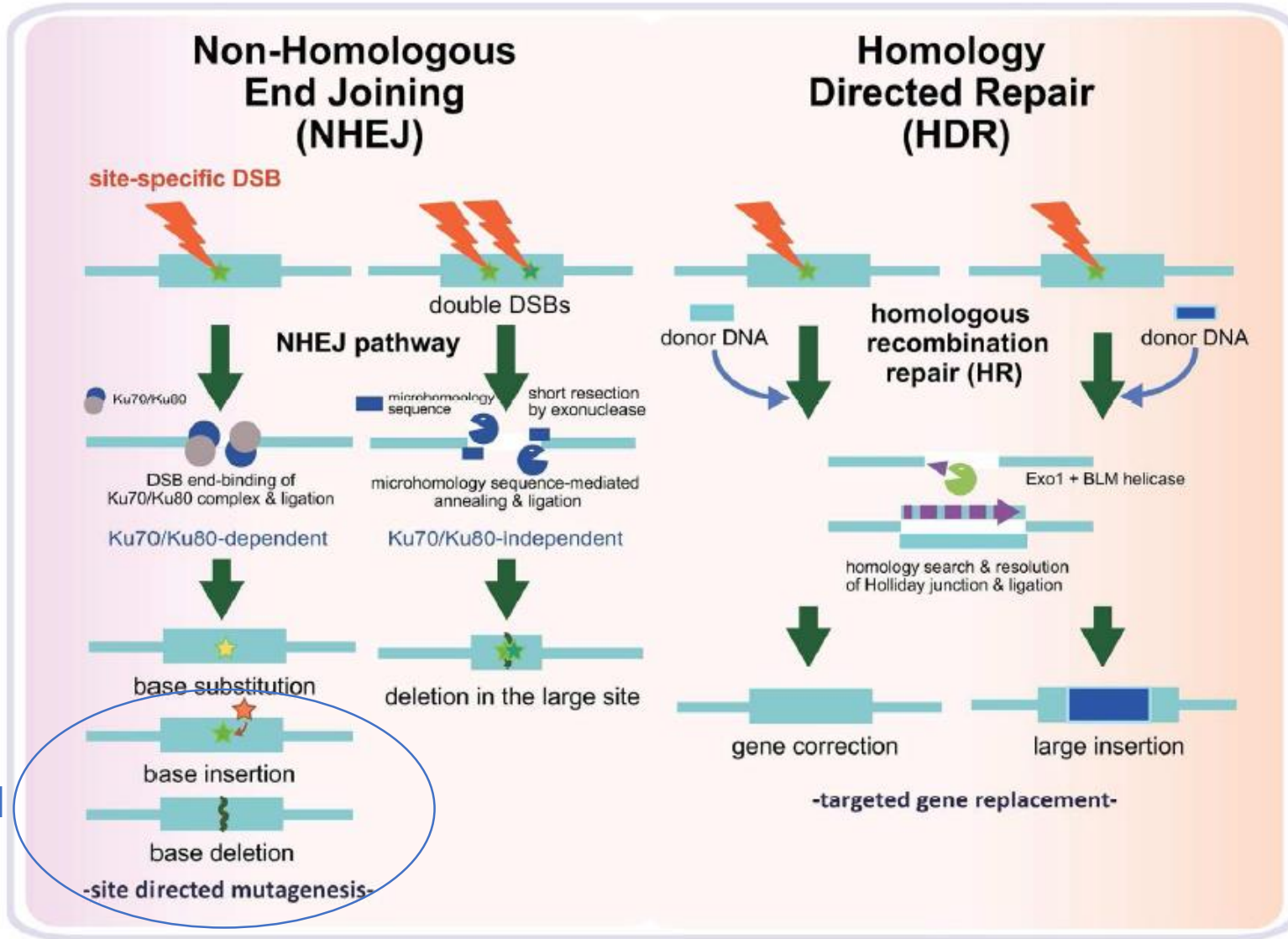


(Zhu et al., 2020)

IMPORTANT:

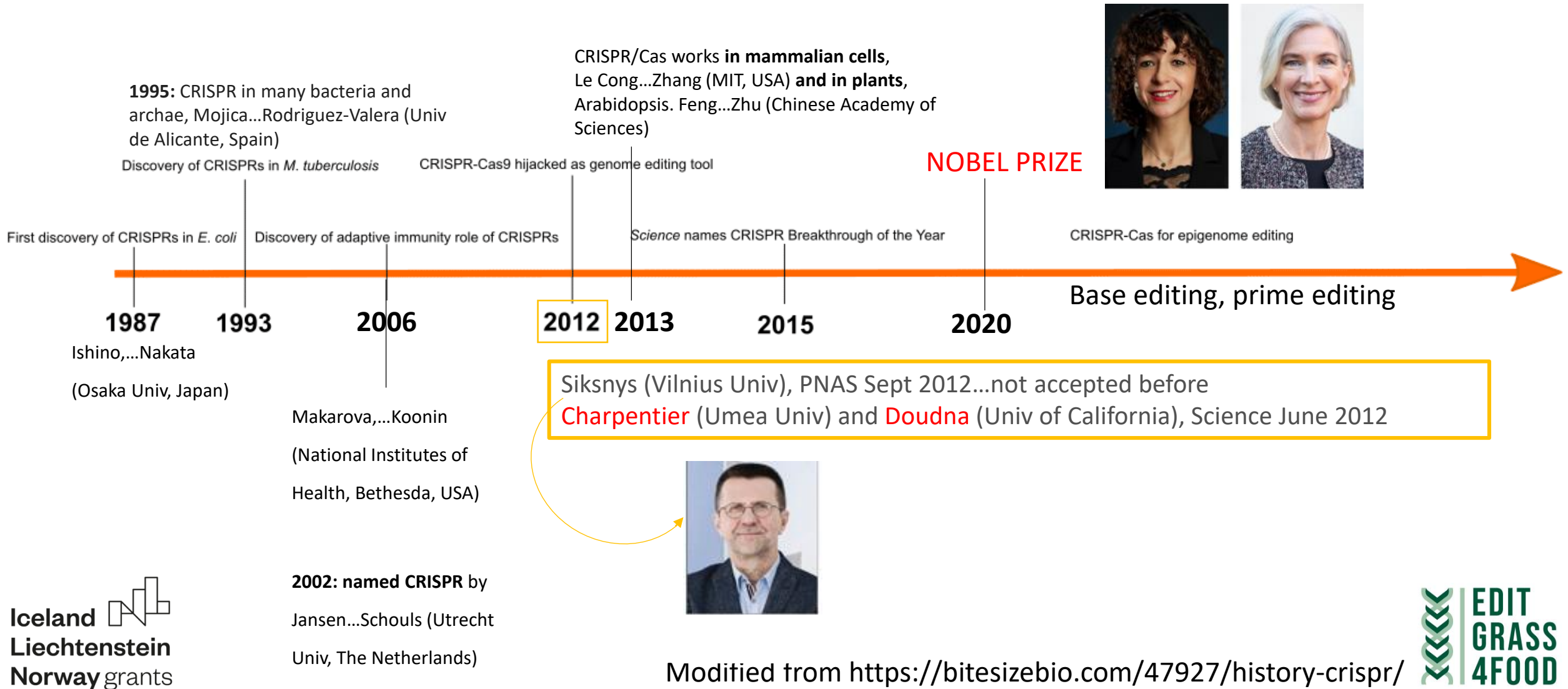
transgenes encoding these components are usually segregated out via sexual crossing

indel

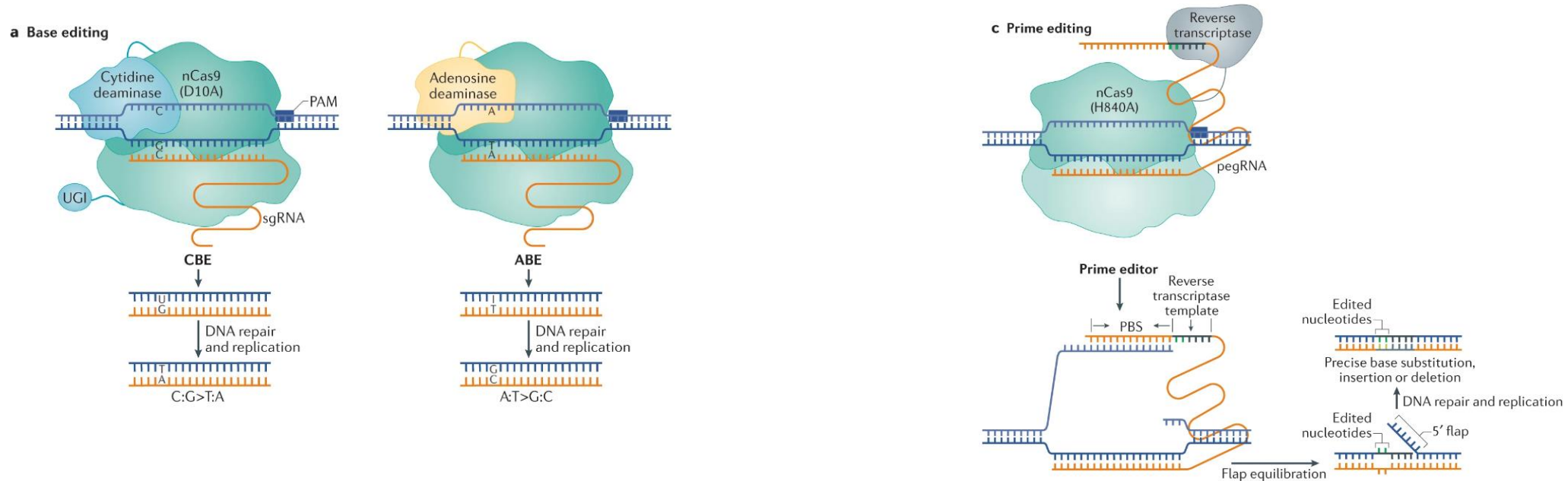


microhomology-mediated end joining (MMEJ)

THE CRISPR/Cas timeline



MANY THINGS CAN BE DONE WITH CRISPR/Cas, not only KO



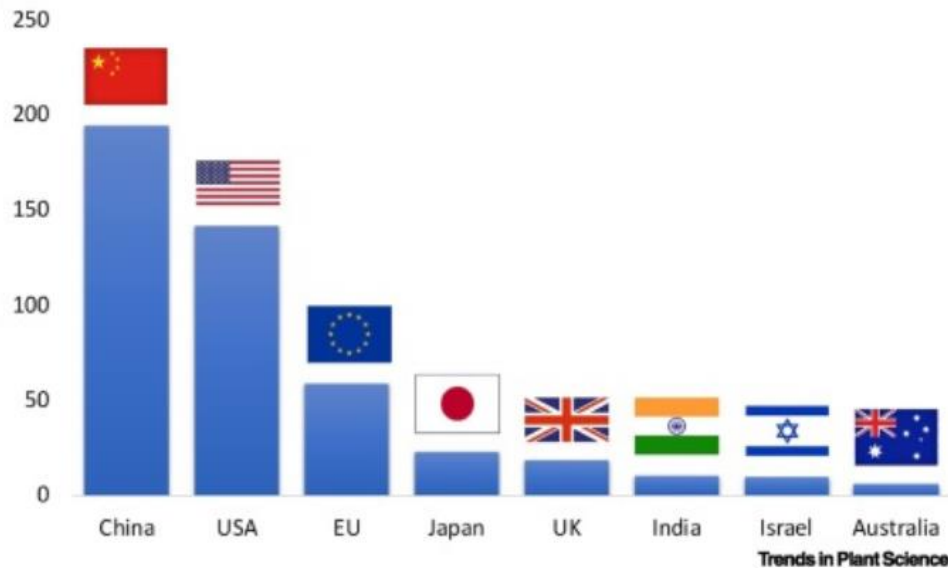
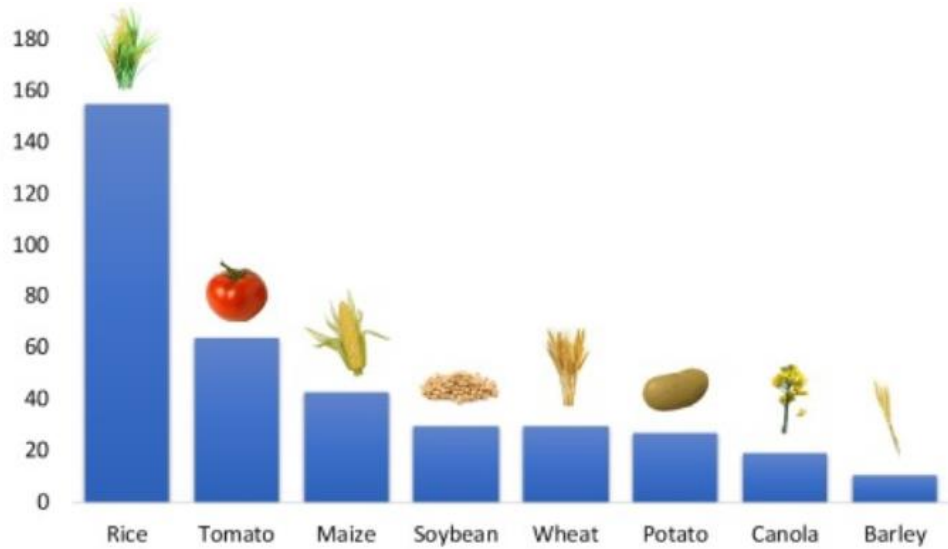
(Zhu *et al.*, 2020)

Modified Cas9 nuclease can perform **targeted epigenome editing**. dead Cas9 (dCas9), can be linked to one of several enzymes that change the epigenome, e.g., DNA demethylases, methylases, or acetyltransferases.

Link modified Cas9 to markers for diagnostics or in situ studies.

While some Cas proteins cleave DNA, others cleave RNA (e.g. Cas13).

WHAT HAS BEEN DONE SO FAR WITH PLANTS?



(Dima et al., 2022)

Most of the genome-edited crops contain specific, small genetic changes that belong to the SDN1 category and are referred to by the EU Commission as 'targeted mutagenesis'. These targeted genetic changes do not differ from the changes that can occur spontaneously in nature or as a result of conventional breeding techniques.



United Soybean Board, better òlis (in the market in the USA since 2019). TALENs



The Sicilian Rouge High GABA tomato (in the market in Japan since 2021)

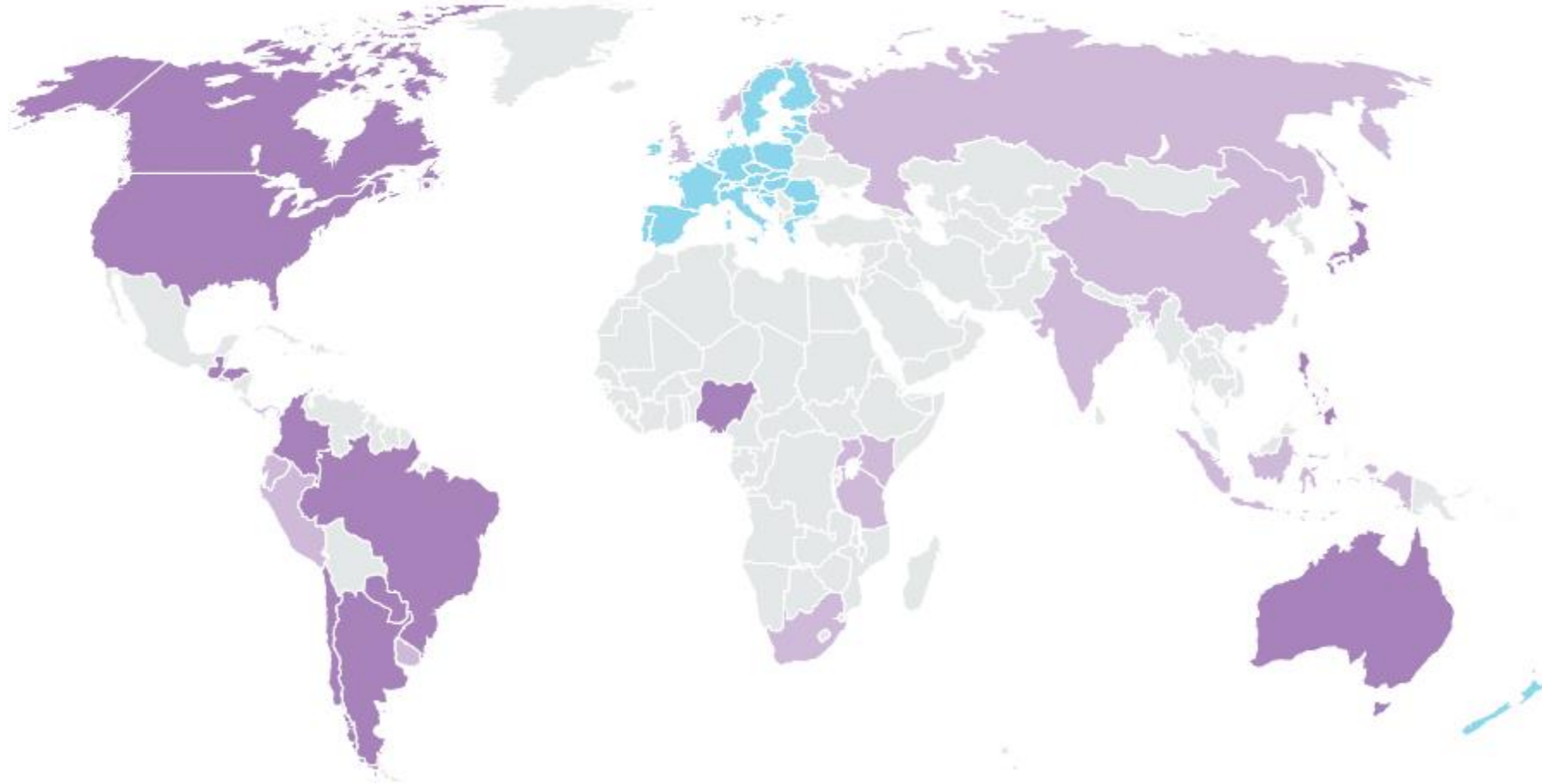
Gene-edited crops in the EU are currently only grown on test fields, for example in Belgium, Spain and Sweden

Trait categories	Description	%
Improved food/feed quality	Modified composition of components such as vitamins, toxic substances, starch, oil, proteins, fibres, allergens, etc. to improve nutritional value.	25
Plant yield and growth	Increased yield related to photosynthetic efficiency, to fruit size or weight, or to increased number of flowers, seeds, and fruits. Improved plant architecture, for example, plant height and shape, growth pattern, and fruit shapes.	22
Biotic stress tolerance	Resistance to plant diseases caused by bacteria, viruses, fungi, pests, pathogens, or nematodes.	18
Industrial utilisation	Applications of industrial interest such as breeding tools, biofuel production, nitrogen use efficiency, etc.	14

Herbicide tolerance 8%, ABIOTIC STRESS TOLERANCE 5%, modified colour or flavour 5%, improvement of storage characteristics 3%

(data until 2021)

(Dima et al., 2022)



-  **Genome-edited crops are not regulated as GMOs**
-  **Discussion is ongoing**
-  **Genome-edited crops are regulated as GMOs**

Organisms obtained by mutagenesis (including genome editing) are GMO



Press and Information

Court of Justice of the European Union

PRESS RELEASE No 111/18

Luxembourg, 25 July 2018

Judgment in Case C-528/16

Confédération paysanne and Others v Premier ministre and Ministre de l'Agriculture, de l'Agroalimentaire et de la Forêt

Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive

However, organisms obtained by mutagenesis techniques which have conventionally been used in a number of applications and have a long safety record are exempt from those obligations, on the understanding that the Member States are free to subject them, in compliance with EU law, to the obligations laid down by the directive or to other obligations

Iceland
Liechtenstein
Norway grants



Brussels, 29.4.2021
SWD(2021) 92 final

COMMISSION STAFF WORKING DOCUMENT

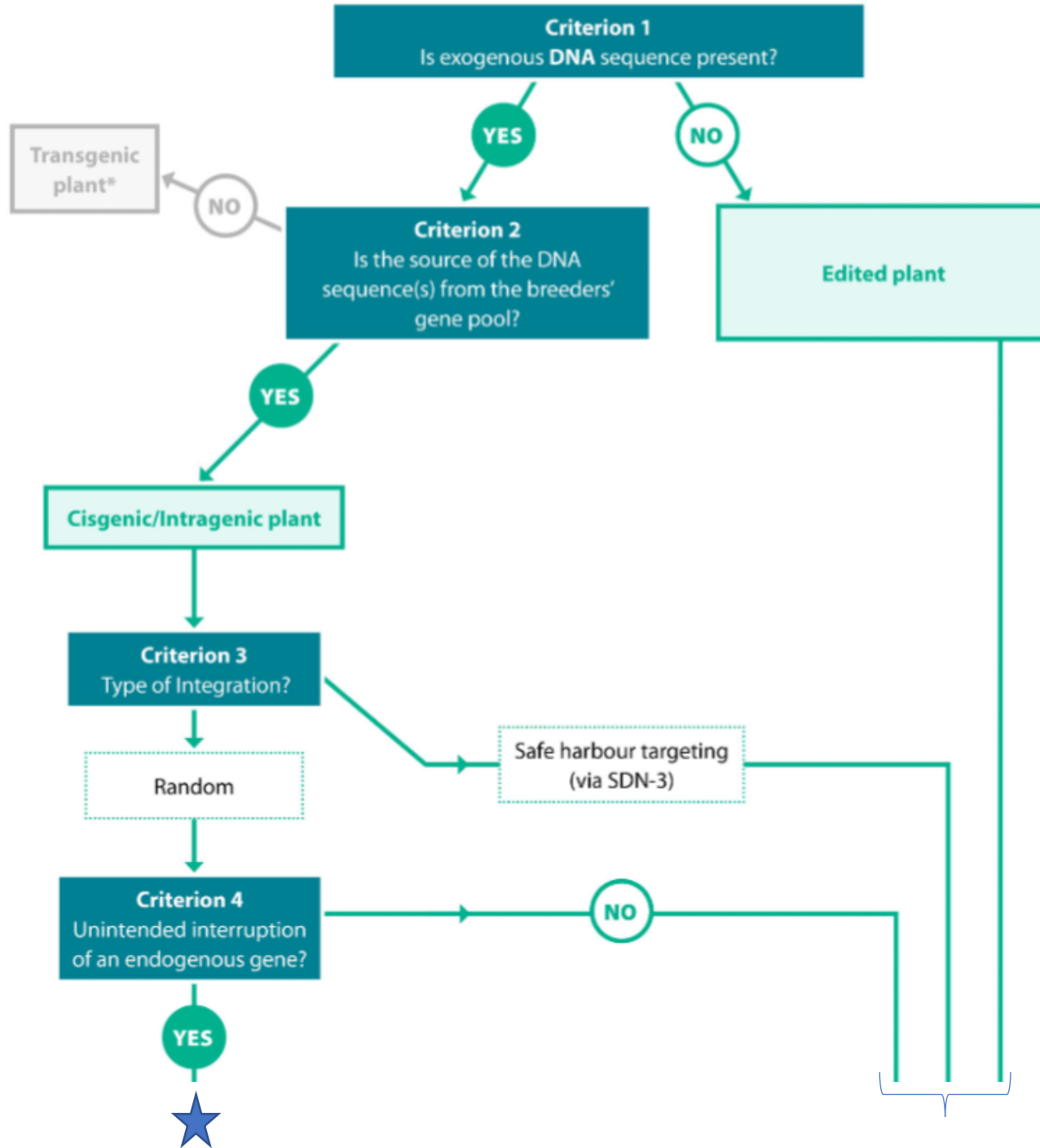
**Study on the status of new genomic techniques under Union law and in light of the
Court of Justice ruling in Case C-528/16**

The Council of the European Union asked for the study, regarding the status of new genomic techniques under Union Law, in light of the Court of Justice's judgment in Case C-528/16

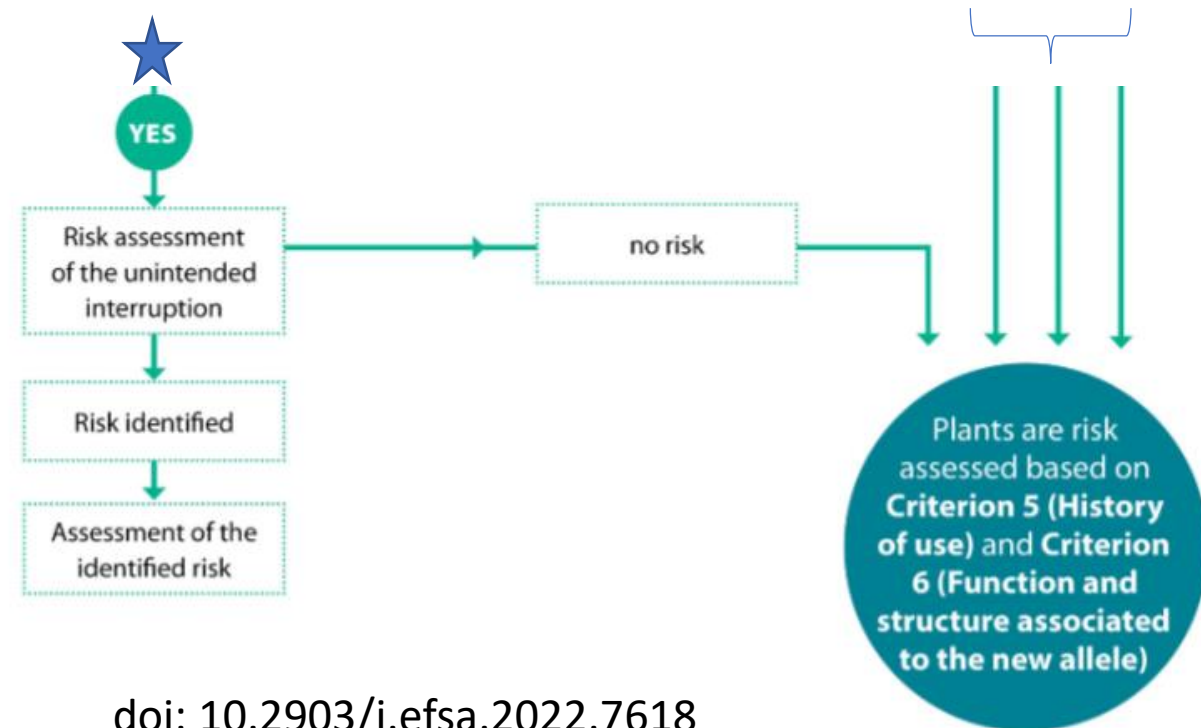
EC STUDY ON NEW GENOMIC TECHNIQUES

Some conclusions of the study:

- There are strong indications that the current GMO legislation is not fit for purpose for some NGTs and their products, and that it needs to be adapted to scientific and technological progress.
- **The Commission also concludes that NGT products have the potential to contribute to sustainable agri-food systems in line with the objectives of the European Green Deal¹² and the 'farm to fork' strategy.** Both of these seek to improve the sustainability of the agri-food system, while also highlighting climate change challenges and noting that biotechnology can play a role, for example, in **reducing dependency on pesticides, developing plants that are more resistant to climatic conditions, as well as contributing to food security and a more sustainable food chain.**
- Drawing generalised conclusions about their safety is impossible. Case-by-case assessment, the study argues, is widely recognised as the appropriate approach.



Upon request from the European Commission, EFSA (European Food Safety Authority) proposed in this statement several **criteria for the risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis**. The criteria proposed should be regarded as **advice** for consideration by the European Commission.
(Published 20th Oct 2022)



doi: 10.2903/j.efsa.2022.7618

BOTTLENECKS FOR CRISPR/Cas TECHNOLOGY

- Transformation
- Tissue culture
- Off-targets
- ...
- **REGULATION IN EU**

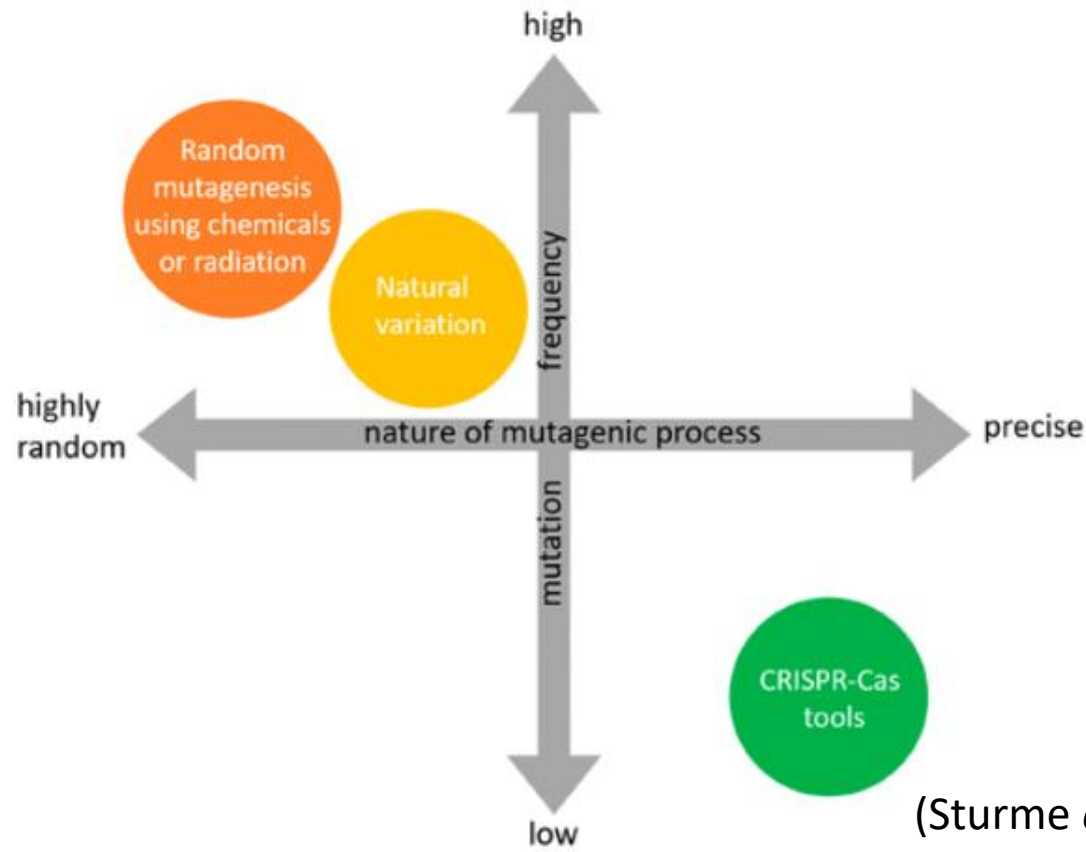
Plants

- Most frequent repair pathway: NHEJ
- Mutation frequency by NHEJ: at least 80% in most species
- Major mutations: short deletions ≤ 10 bp or 1-bp insertions (A/T)
- Insertion frequency by HDR: ~ 0.2 – 5.5%
- Off-target mutations: rare

Animals

- Most frequent repair pathway: NHEJ
- Mutation frequency by NHEJ: at least 90% in most species
- Major mutations: short deletions ≤ 40 bp
- Insertion frequency by HDR: ~ 5 – 20%
- Off-target mutations: rare, but higher in cancer cells

Modified from Bortesi *et al.* (2016)



(Sturme *et al.*, 2022)

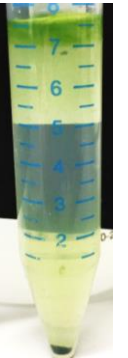
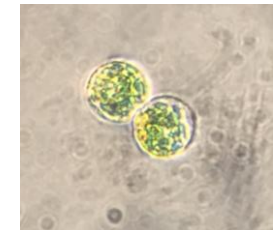
OUR WORK (TALTECH)

- ❖ Tissue culture of *Lolium perenne*
- ❖ CRISPR/Cas9 work

CANDIDATE GENES FOR CRISPR KNOCK OUT

Gene	Denmark genome homologue	Identity	Coverage	Query
VIN3	Lp_chr4_0G13152.1		100	99.7 Lolium Mallik
QM	Lp_chr1_0G4562.1		89.3	90.9 Hordeum predicted
	Lp_chr3_0G20332.1		88.6	93.3 Hordeum predicted
IRI1	Lp_chr3_0G9338.1		96.1	100 Lolium perenne
	Lp_chr5_0G782.1		96.1	100 Lolium perenne
CBF6	Lp_chr5_0G16922.1		81	93.3 Hordeum predicted
DHN1	Lp_chr1_0G4004.1		92.4	53.3 Lolium Mallik
EF-CAX	Lp_chr2_0G4306.1	FROST	98.9	100 Lolium partial mRNA
	Lp_chr3_0G7610.1		86.3	93.8 Hordeum predicted
	Lp_chr6_0G8564.1		90.8	77.9 Hordeum predicted
GI	Lp_chr3_0G8870.1		90.3	92.9 Hordeum predicted
LEA-14	Lp_chr3_0G15594.1		90.3	66.2 Hordeum predicted
PRR95	Lp_chr5_0G17244.1		79	70.6 Oryza sativa
TPT	Lp_chr3_0G9158.1		87.4	100 Hordeum predicted
VRN1	Lp_chr4_0G5312.1		98	100 Lolium Mallik
CBP20	Lp_chr6_0G11740.1		93	97.8 Hordeum

- ❖ Vectors: for introducing 1-6 gRNAs.
- ❖ Modular vectors for easy cloning.
- ❖ GRF-GIF chimera for efficient regeneration.
- ❖ Test the sgRNAs in protoplasts



❖ Design of gRNAs

❖ **CRISPOR**: using SWISS GENOME for off-targets

❖ **GENIOUS**: using DANISH GENOME for off-targets

Gene	Denmark genome homologue	Identity	Coverage	Query
PHYB	Lp_chr4_0G21910.1	97.5	100	Lolium perenne
	Lp_chr4_0G21912.1	97.5	100	Lolium perenne
MYB41	Lp_chr2_0G18342.1	98.1	100	Lolium rigidum predicted
NAC038	Lp_chr5_0G15838.1	82.7	67.3	Hordeum vulgare morex
MYB94	Lp_chr2_0G4322.1	96.7	70.4	Lolium rigidum predicted
WSD11 /FOP1	Lp_chr2_0G20.1	94.2	100	Lolium rigidum predicted
TSO1	Lp_chr2_0G11088.1	83.8	90.4	Hordeum predicted
MYB4	Lp_chr2_0G17906.1	94.6	94.9	Lolium rigidum predicted
HSL1	Lp_chr5_0G12878.1	82.3	89.7	Hordeum predicted
WRKY49	Lp_chr3_0G17844.1	85.3	74.4	Hordeum vulgare
	Lp_chr3_0G17848.1	83.1	88	Hordeum vulgare
PRR5	Lp_chr1_0G13020.1	68	40	Hordeum predicted

DROUGHT

No	Type	Country of origin	Cultivar name	Drought sensitivity
22	cultivar	RU	Leningradskij 809	tolerant
3177	ecotype	LT		tolerant
3618	cultivar	FI	Riikka	tolerant
3943	cultivar	NL	Kerdion	moderate
3948	cultivar	NL	Magella	moderate
3938	cultivar	NZ	Grasslands Nui	moderate-sensitive
3944	cultivar	DE	Kerem	moderate-sensitive
34551	ecotype			moderate-sensitive
3774	ecotype	UA		moderate-sensitive
3784	ecotype	LV		moderate-sensitive
3821	ecotype	UA		sensitive
3575	ecotype	LT		sensitive
3776	ecotype	SK		sensitive

From tillers



From seeds



THANK YOU FOR YOUR ATTENTION!

PlantEd

COST Action CA18111 – Genome editing in plants



European Plant Science Organisation



MAAELUMINISTEERIUM
(Uute aretustehnikate
töörühm)

eusage

European Sustainable Agriculture
Through Genome Editing

Ferenz Sustek
Erki Eelmets
Olav Kasterpalu
Merike Sõmera
Lenne Nigul
Signe Nõus



Iceland
Liechtenstein
Norway grants

