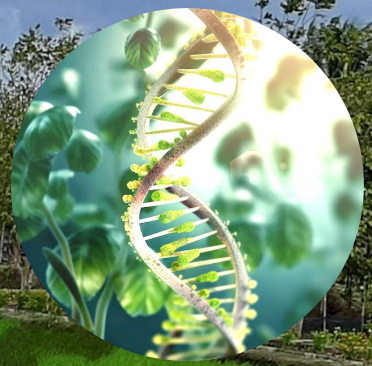
New Technologies Driving the Future of Plant Breeding



REYNANTE L. ORDONIO, PhD
Scientist I
Philippine Rice Research Institute

Webinar series: Biotech Innovations for a Sustainable Agriculture
November 8, 2023

Scope of the Presentation

1. New wave of plant breeding innovations and their potential impact in the future;
2. Overview of the current global regulatory frameworks

1. Advanced genome/transcriptome sequencing or genotyping tools (e.g., NGS systems like Illumina, Pacbio, Nanopore)

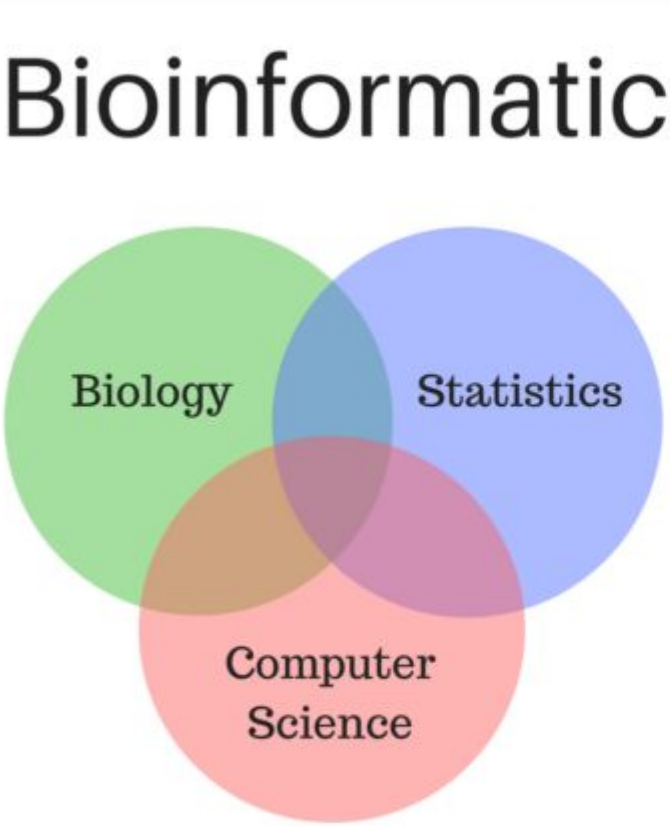
MiSeq

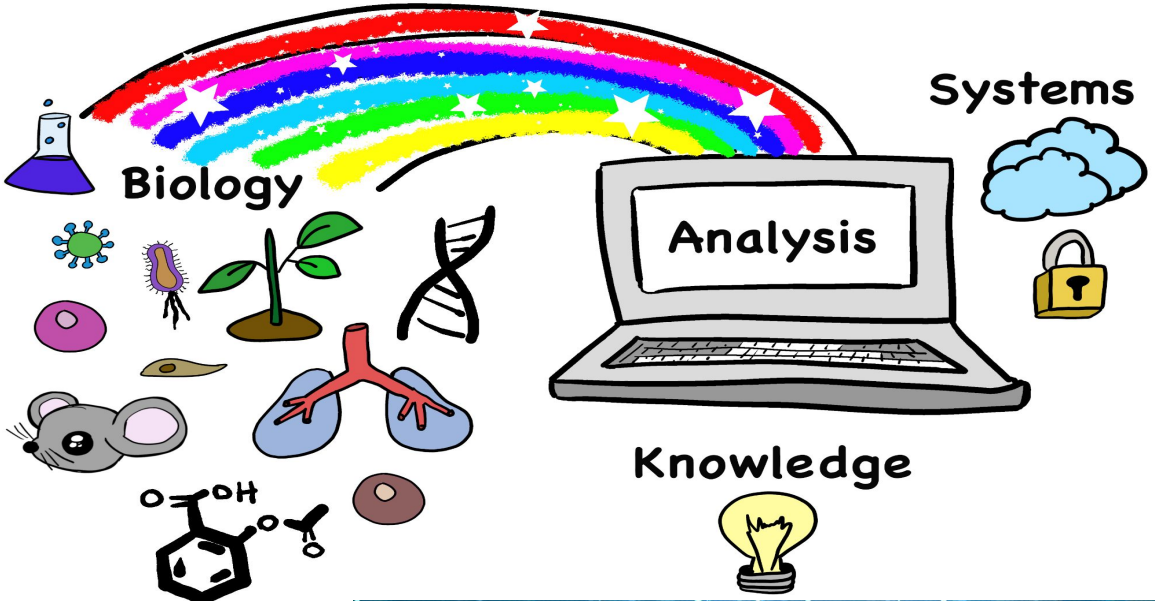
NextSeq 500

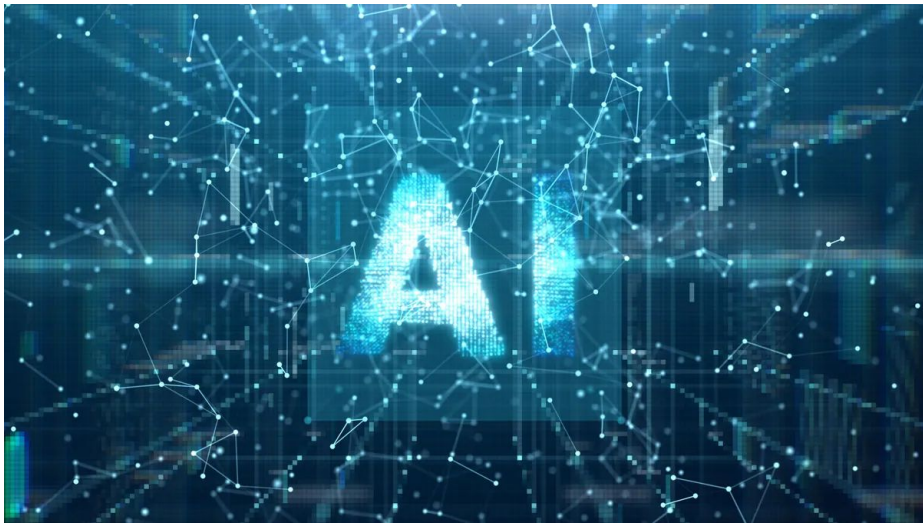
AVITI

NovaSeq 6000

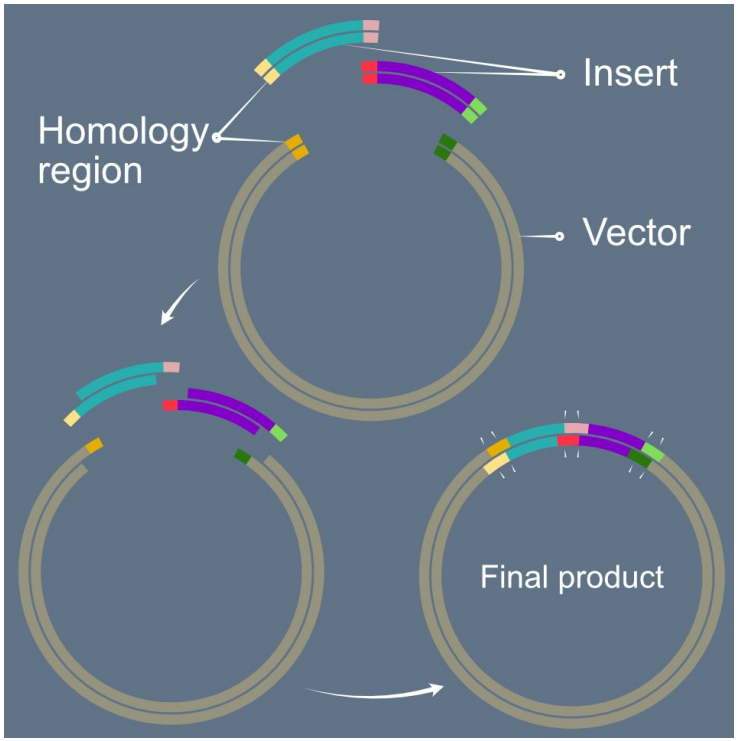
2. Advanced data analytical tools (e.g., bioinformatics, AI)





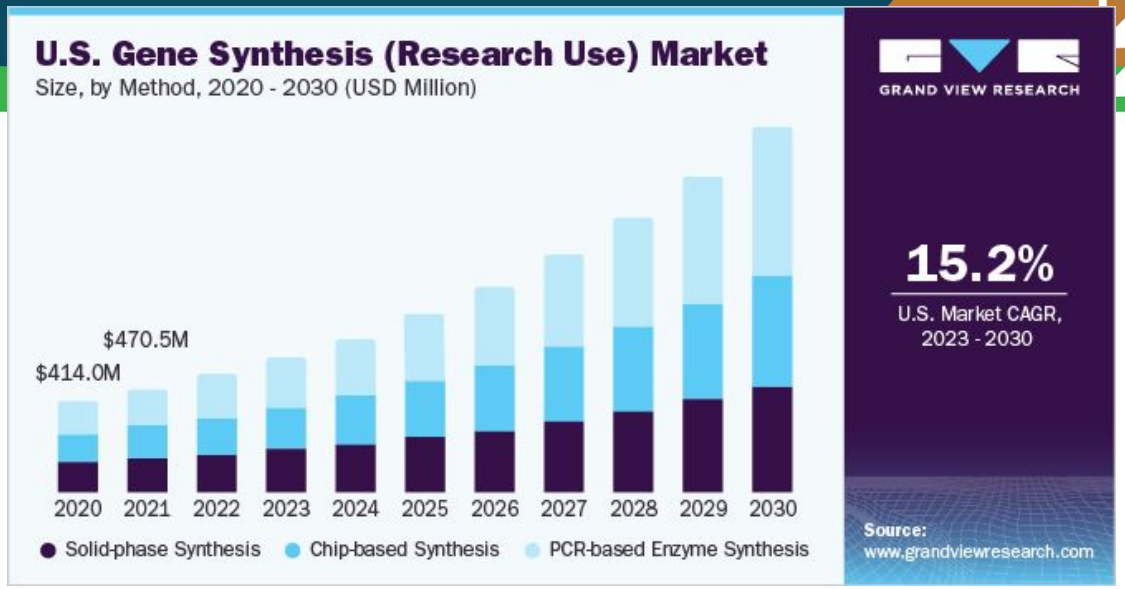


3. Advanced DNA synthesis technologies (up to genome level)

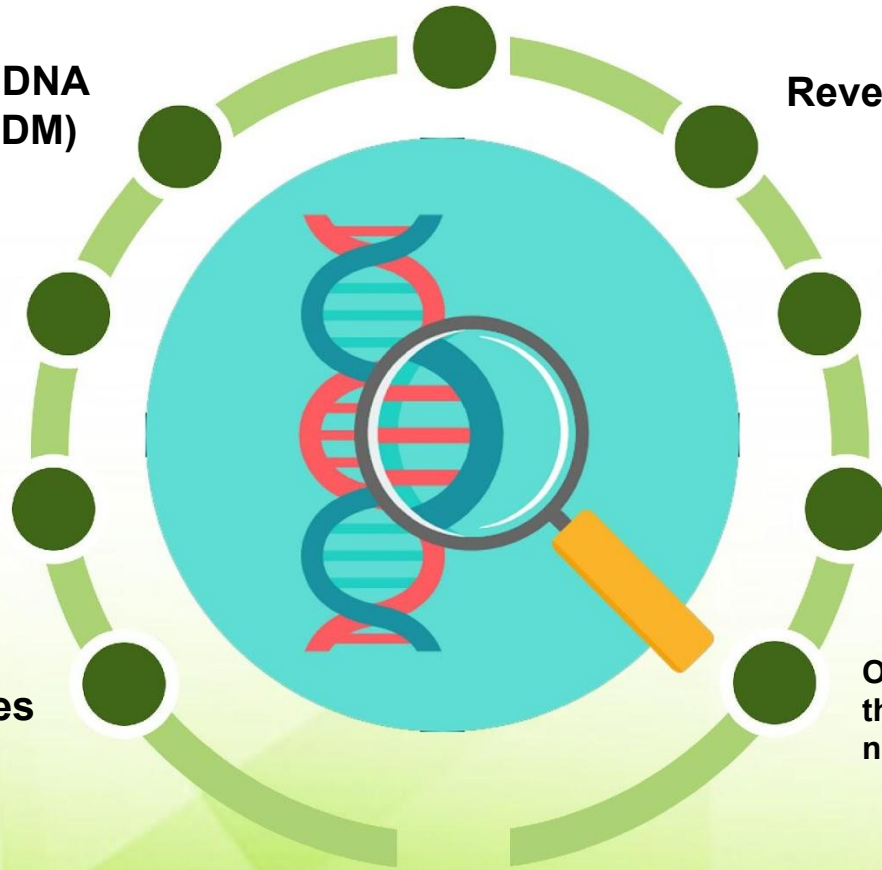


Gibson Assembly

a.k.a Gibson DNA assembly or Gibson cloning



4. Plant Breeding Innovations (including Precision Breeding)



Grafting with GM material

RNA-dependent DNA Methylation (RdDM)

Reverse Breeding

Cisgenesis and Intragenesis

Agro-infiltration

Oligonucleotide-Directed Mutagenesis (ODM)

Synthetic Genomics

Site-Directed Nucleases (SDN)

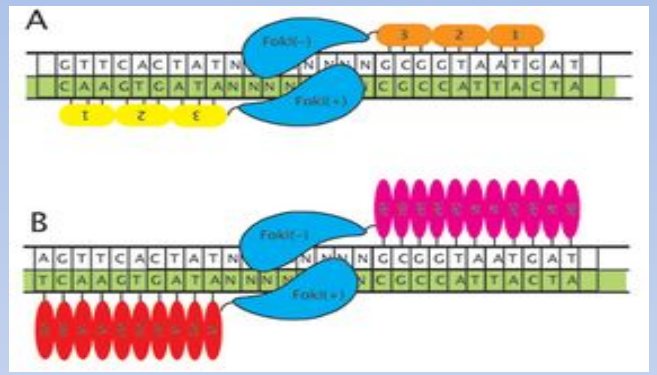
Other or upcoming techniques that have the potential to produce non-GM or both non-GM and GM plants as final products

Plant Breeding Innovations

1. Site-Directed Nucleases or SDN (e.g., ZFN, TALEN, CRISPR-Cas)

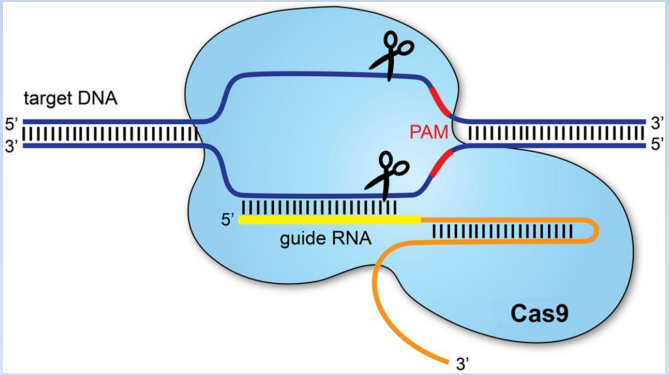
ZFN

TALEN



https://www.addgene.org/talen/guide/

CRISPR-Cas9

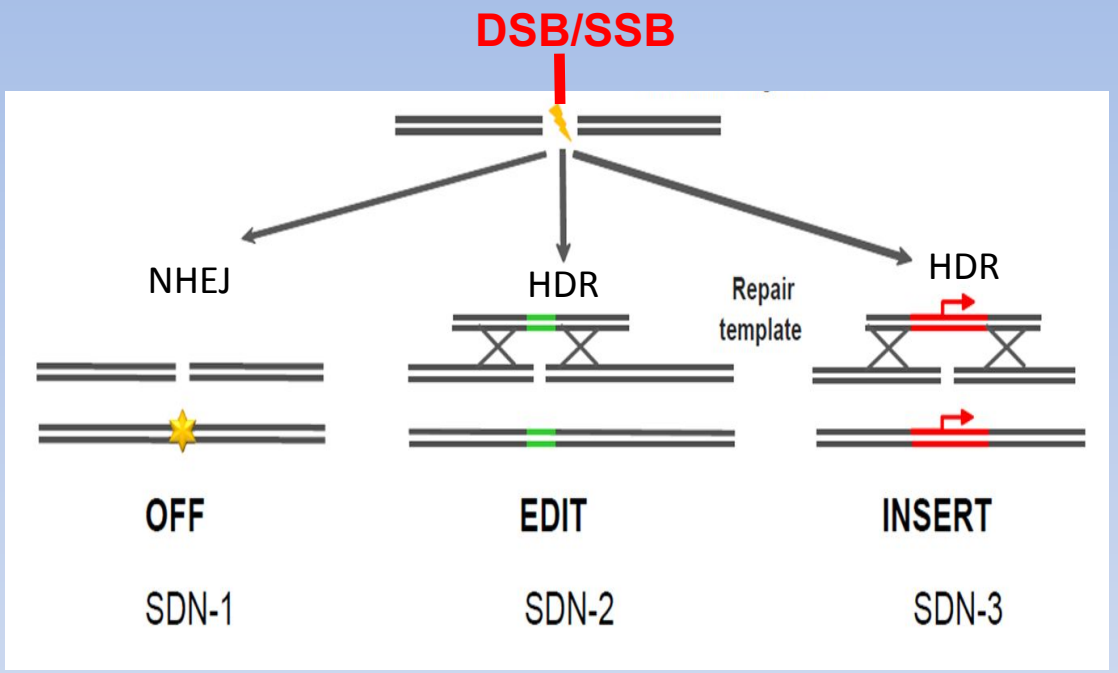


https://ep.bmj.com/content/101/4/213

- DNA-cutting enzyme (nuclease) that is directed to cleave the DNA at a predetermined location as guided by a specific DNA binding component, producing a DSB or SSB.
- USE: Introduction of a targeted mutation (e.g., insertion or deletion) in a precise location (in the DSB or SSB).

Plant Breeding Innovations

Repair of the double-strand break (DSB)



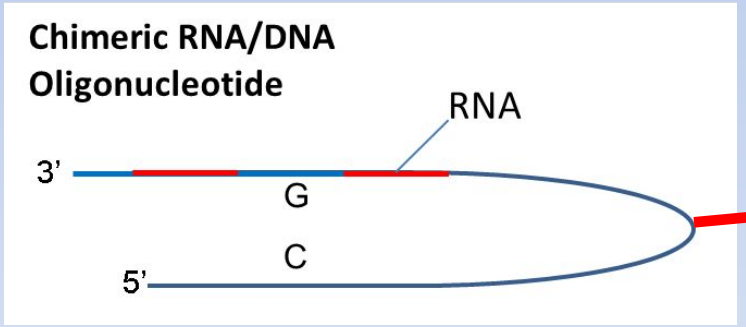
Non-GMO | Non-GMO | Case by case

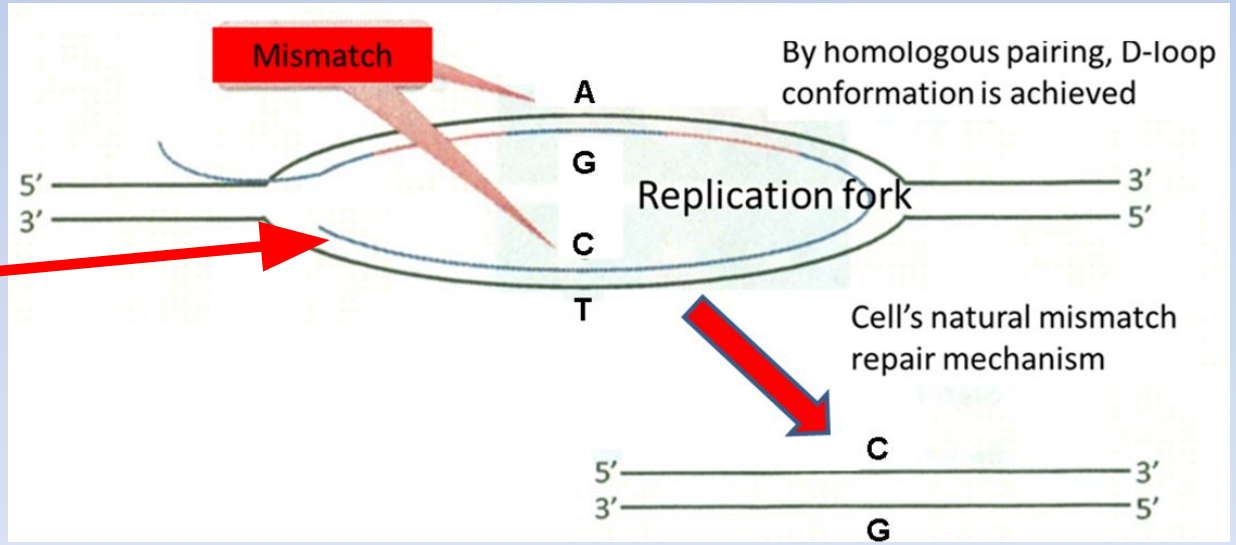
- DSB repair method and nature of insert determine if product is GMO or not
- SDN1 – no insertion, no repair template; non-GMO*
- SDN2 – insertion of 19 bp and below; non-GMO
- SDN3 – insertion of 20 bp and above; case-by-case

*Need to breed out any vector sequences

2. Oligonucleotide-directed mutagenesis (ODM)

- Uses customized single-stranded oligonucleotides to interfere with DNA replication at the replication fork within the target gene, resulting in the introduction of desired bases (hence, mutation) into the DNA.
- USE: Introduction of a non-random mutation (e.g., base change) in a precise location to which the designed oligonucleotide is complementary.
- Non-GMO as product

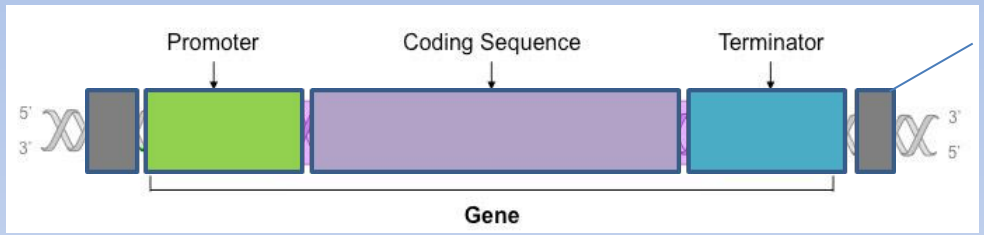




3. Cisgenesis and Intragenesis

- Cisgenesis: genetic transformation of a recipient plant with a natural gene (allele) from a crossable (sexually compatible) plant.

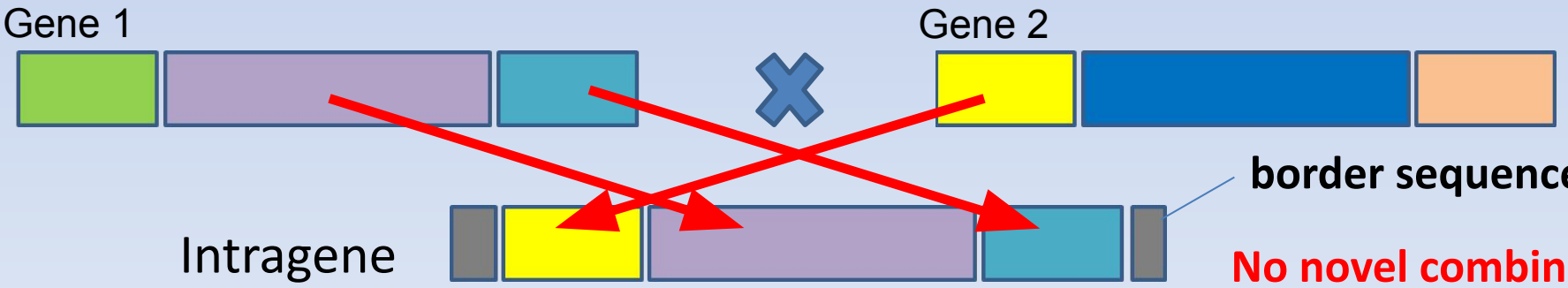
Cisgene



border sequence (<20bp)

No novel combination

- Intragenesis: Introduces an altered gene (different choice of promoters, exons and introns) from the same or cross-compatible species

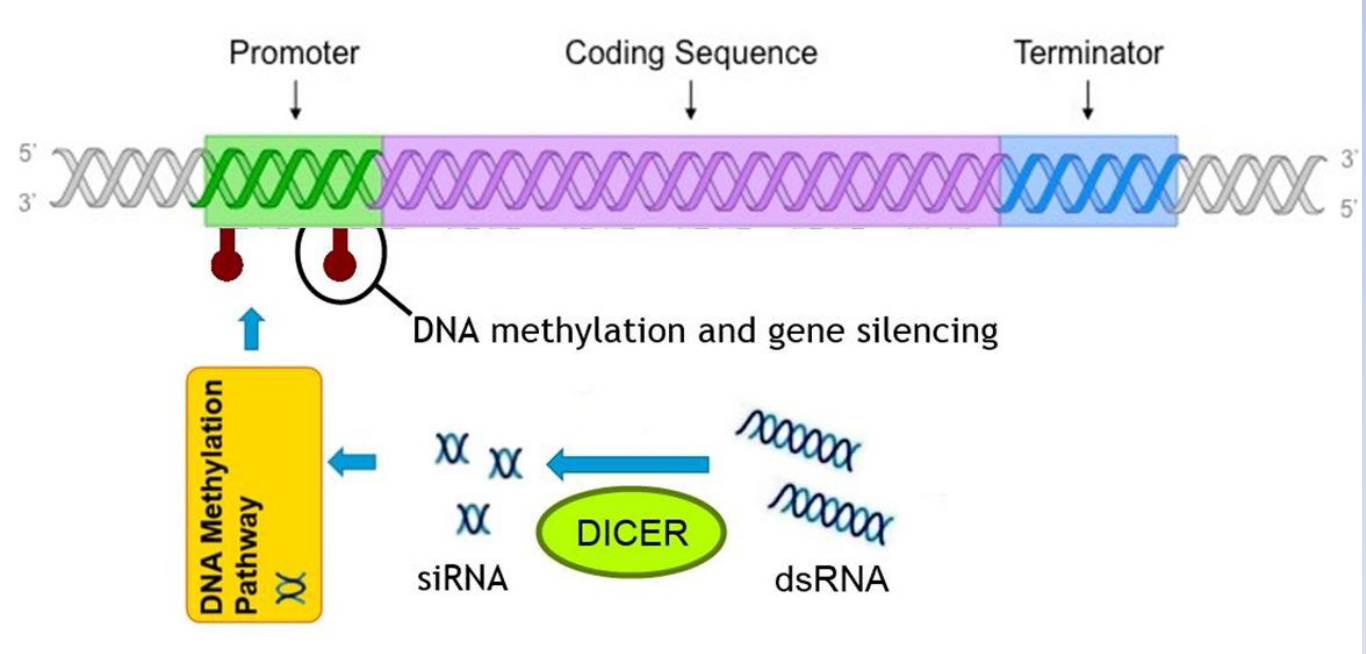


4. RNA-dependent DNA methylation (RdDM)

- Knocks down (silences) target genes through RNAi, resulting in the attachment of a -CH3 to their promoter sequence (epigenetic control)

- Methylation does not change the DNA sequence

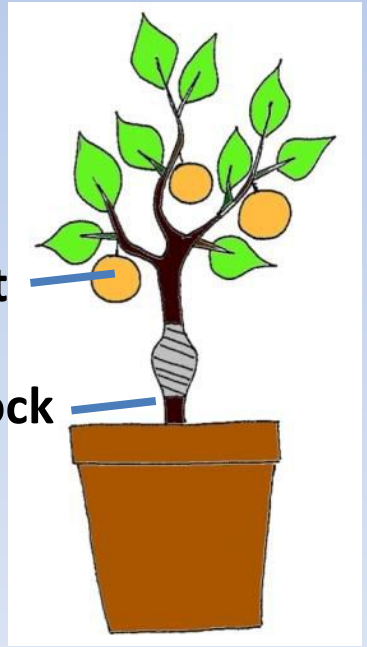
- Null segregants as final products (non-GMOs)

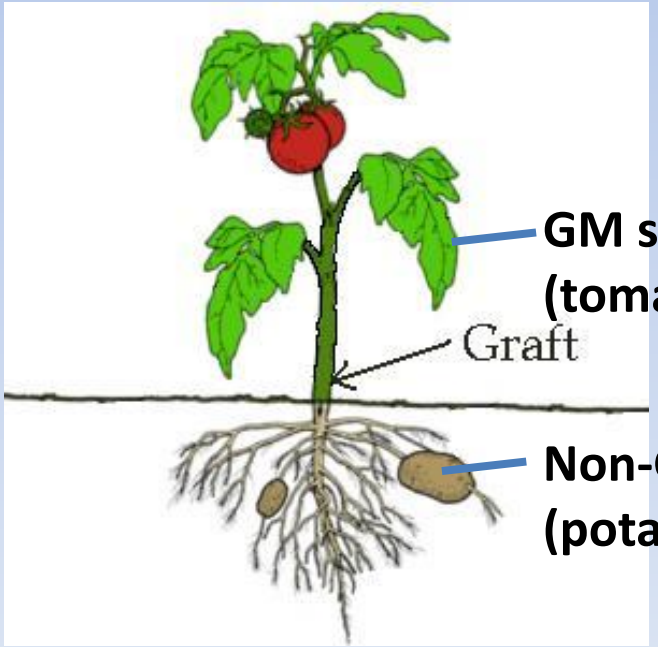


Plant Breeding Innovations

5. Grafting with GM Material

- Fusing together a rootstock and scion, either one being a GMO
- Produces non-GMO products from the non-GMO scion or root stock
- Essential molecules are translocated from the transgenic part but not DNA

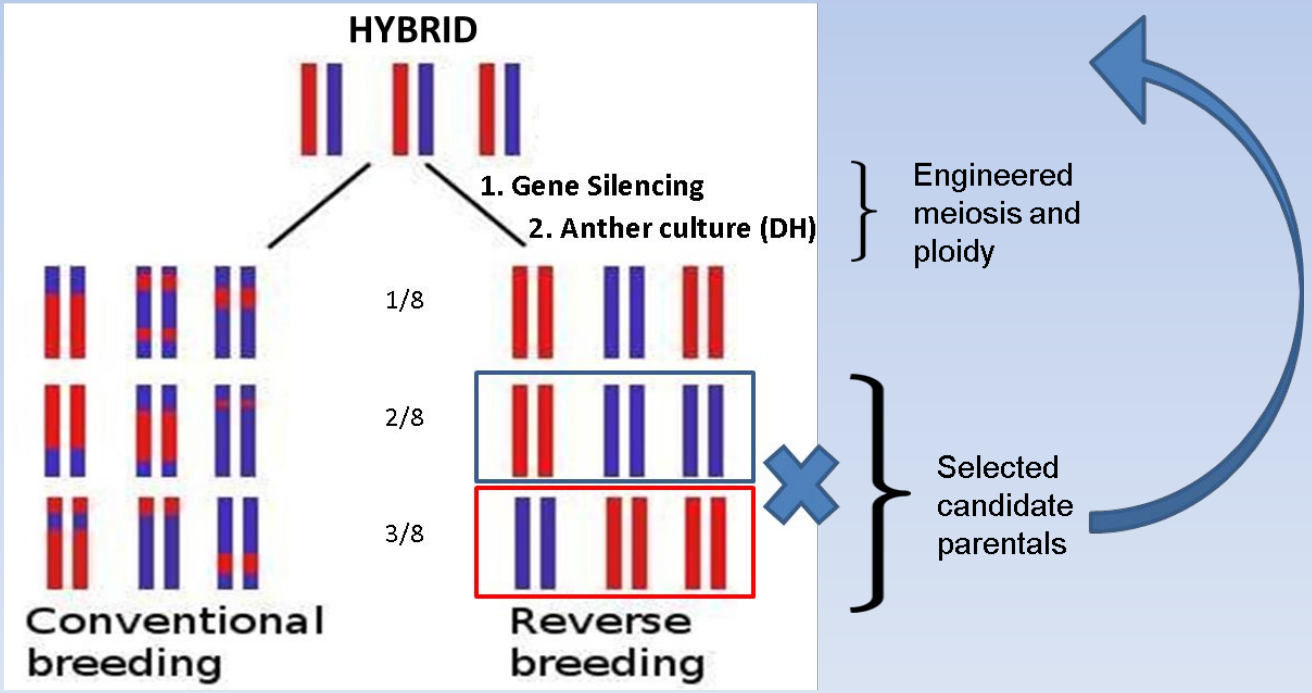




Plant Breeding Innovations

6. Reverse breeding

- Prevents “crossing over” in the hybrid (of unknown parentage) during meiosis through RNAi
- Produces homozygous candidate parentals (null segregants) as final products (non-GMO)

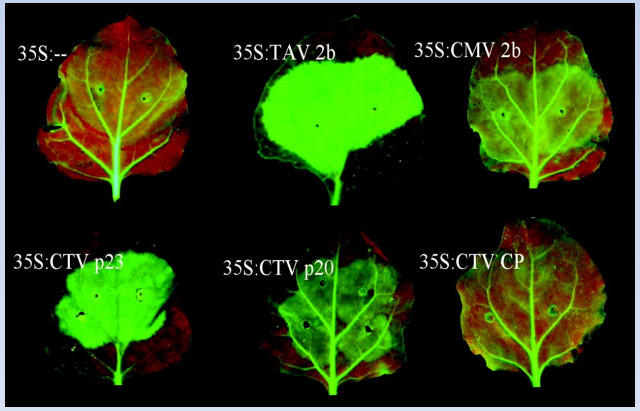


Plant Breeding Innovations

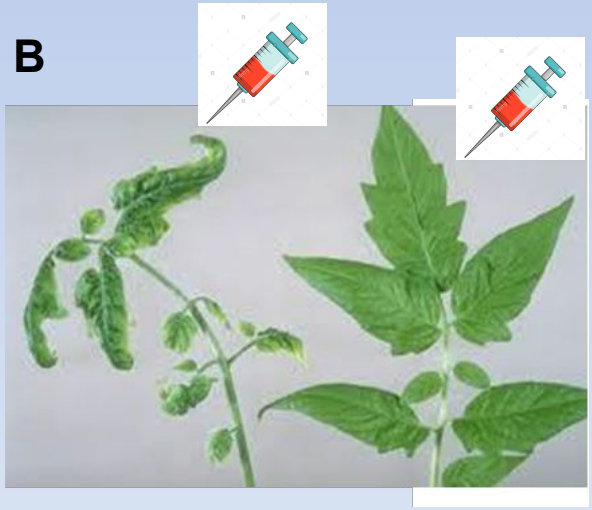
7. Agro-infiltration – *Agrobacterium tumefaciens*-mediated

- **Non-germline (Agroinoculation):** Test a construct (transient expression) (A) or to express virus particles to screen for resistant plants (B), without affecting the germline tissues of the host
- **Germline (Floral dip):** Insertion of a gene through the floral parts, immature embryo or meristems that will give rise to seeds (C)

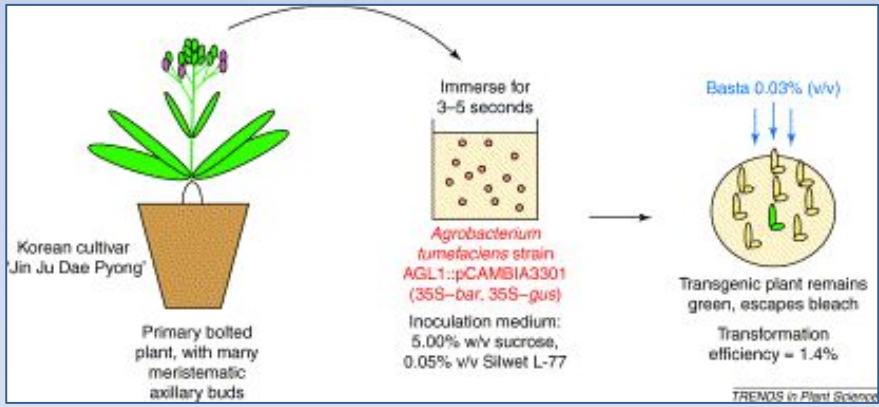
A



B



C



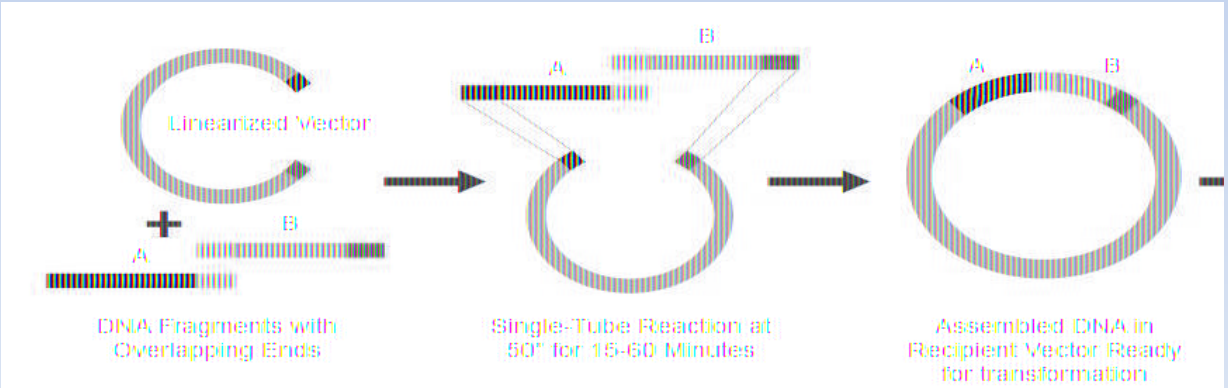
A. Non-germline – no novel combination

B. Germline (Floral Dip) – case by case

Plant Breeding Innovations

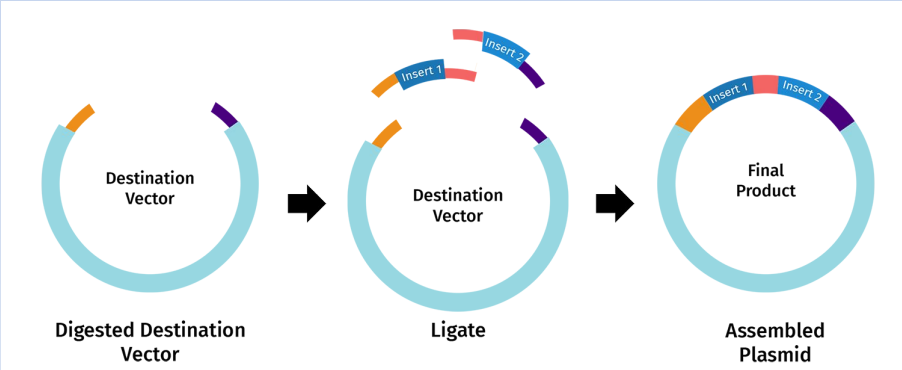
8. Synthetic Genomics (*overlaps with Synthetic Biology*)

- Engineering and manipulation of an organism's genetic material on the scale of the whole genome, based on technologies to design and chemically synthesize pieces of DNA (e.g., Gibson and Golden Gate Assembly) and to assemble them to long, chromosome-sized fragments *(Konig et al., 2013)*
- As an NBT, it can create a non-GMO by faithfully duplicating the genome
- Can also create a GMO



Gibson Assembly

Raimbault et al. 2016; Gibson et al. 2008



Golden Gate Assembly (1996)

IRRI leads the way in rice gene editing in PH

Plant Cell Rep. 2017 May;36(5):745-757. doi: 10.1007/s00299-017-2118-z. Epub 2017 Mar 27.

CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene EPFL9 in rice

Xiaojia Yin [1], Akshaya K Biswal [1 2], Jacqueline Dionora [1], Kristel M Perdigon [1], Christian P Balahadia [1], Shamik Mazumdar [1], Caspar Chater [3 4], Hsiang-Chun Lin [1], Robert A Coe [1], Tobias Kretzschmar [1], Julie E Gray [3], Paul W Quick [1 5], Anindya Bandyopadhyay [6]

2017

Plant Biotechnol J. 2018 Nov;16(11):1918-1927. doi: 10.1111/pbi.12927. Epub 2018 Apr 30.

Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus

Anca Macovei [1], Neah R Sevilla [1], Christian Cantos [1], Gilda B Jonson [1], Inez Slamet-Loedin [1], Tomáš Čermák [2], Daniel F Voytas [2], Il-Ryong Choi [1], Prabhjit Chadha-Mohanty [1]

2018

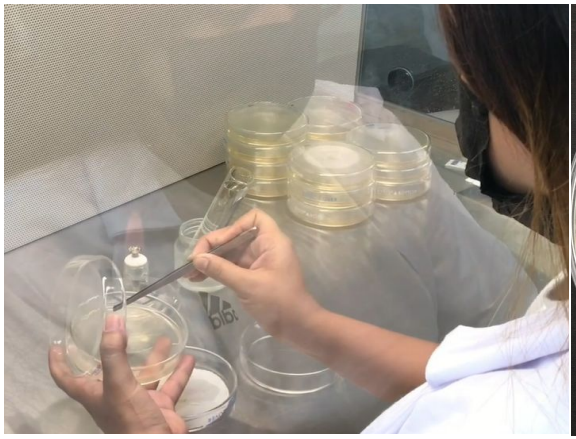
Broad-spectrum resistance to bacterial blight in rice using genome editing

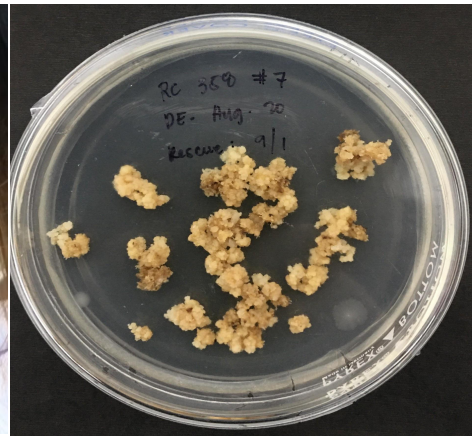
Ricardo Oliva, Chonghui Ji, Genelou Atienza-Grande, José C. Huguet-Tapia, Alvaro Perez-Quintero, Ting Li, Joon-Seob Eom, Chenhao Li, Hanna Nguyen, Bo Liu, Florence Auguy, Coline Sciallano, Van T. Luu, Gerbert S. Dossa, Sébastien Cunnac, Sarah M. Schmidt, Inez H. Slamet-Loedin, Casiana Vera Cruz, Boris Szurek, Wolf B. Frommer, Frank F. White & Bing Yang

Nature Biotechnology **37**, 1344–1350 (2019) | Cite this article

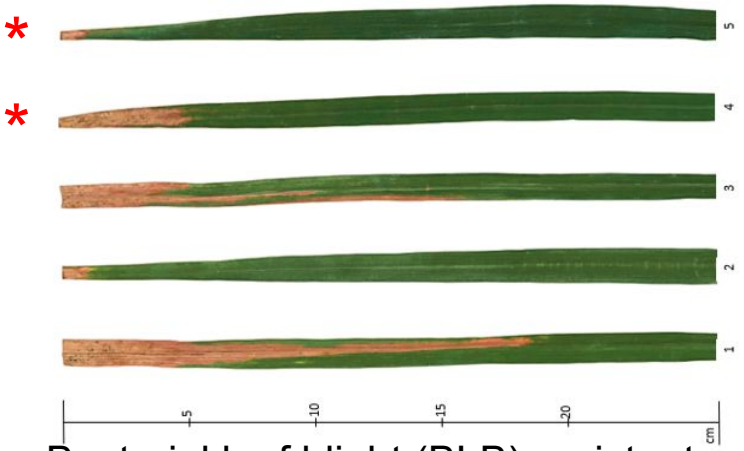
2019

Capacitating PhilRice for genome editing research





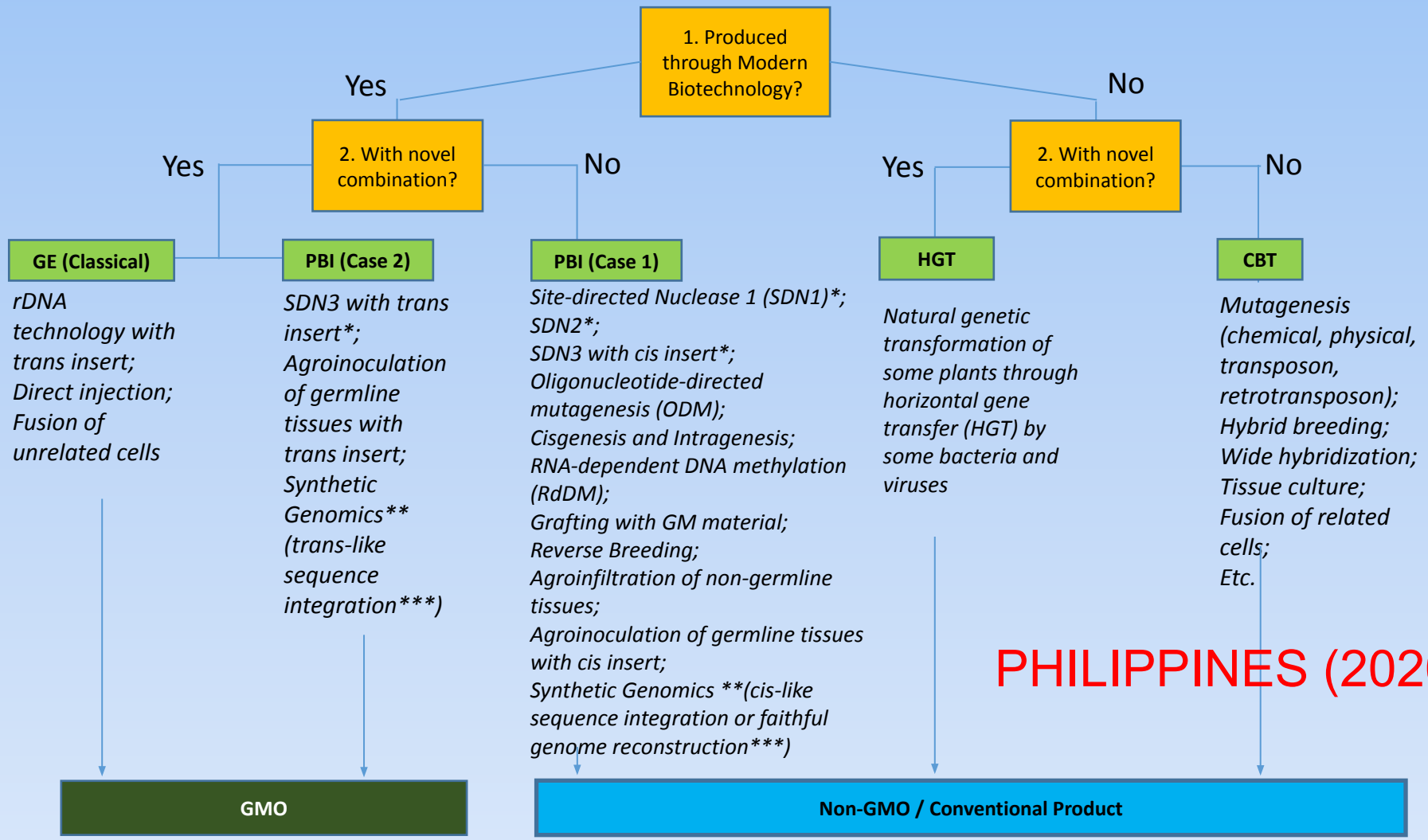
RTSV-resistant rice lines (*eIF4g*)



Bacterial leaf blight (BLB)-resistant rice lines (*OsSweet14 and 13*)

Overview of the current global regulatory frameworks on PBIs

Decision tree for the classification of plant and plant products derived from Plant Breeding Innovations (PBI) ANNEX A



PHILIPPINES (2020)

Note: *Techniques listed under PBI Case 1 and Case 2 may expand as new technologies emerge. Any PBI technique must be able to produce a non-GM or both non-GM and GM plant as a final product..*

*Includes the new CRISPR-Cas9-based Prime Editing (Anzalone et al., 2019) ** Different from but converges with Synthetic Biology, which specializes on sequences/genetic elements (e.g. unnatural base pairs) in the genome that are not found in nature (beyond novel combination). ***Pertains to a largely synthetic assembled genome.

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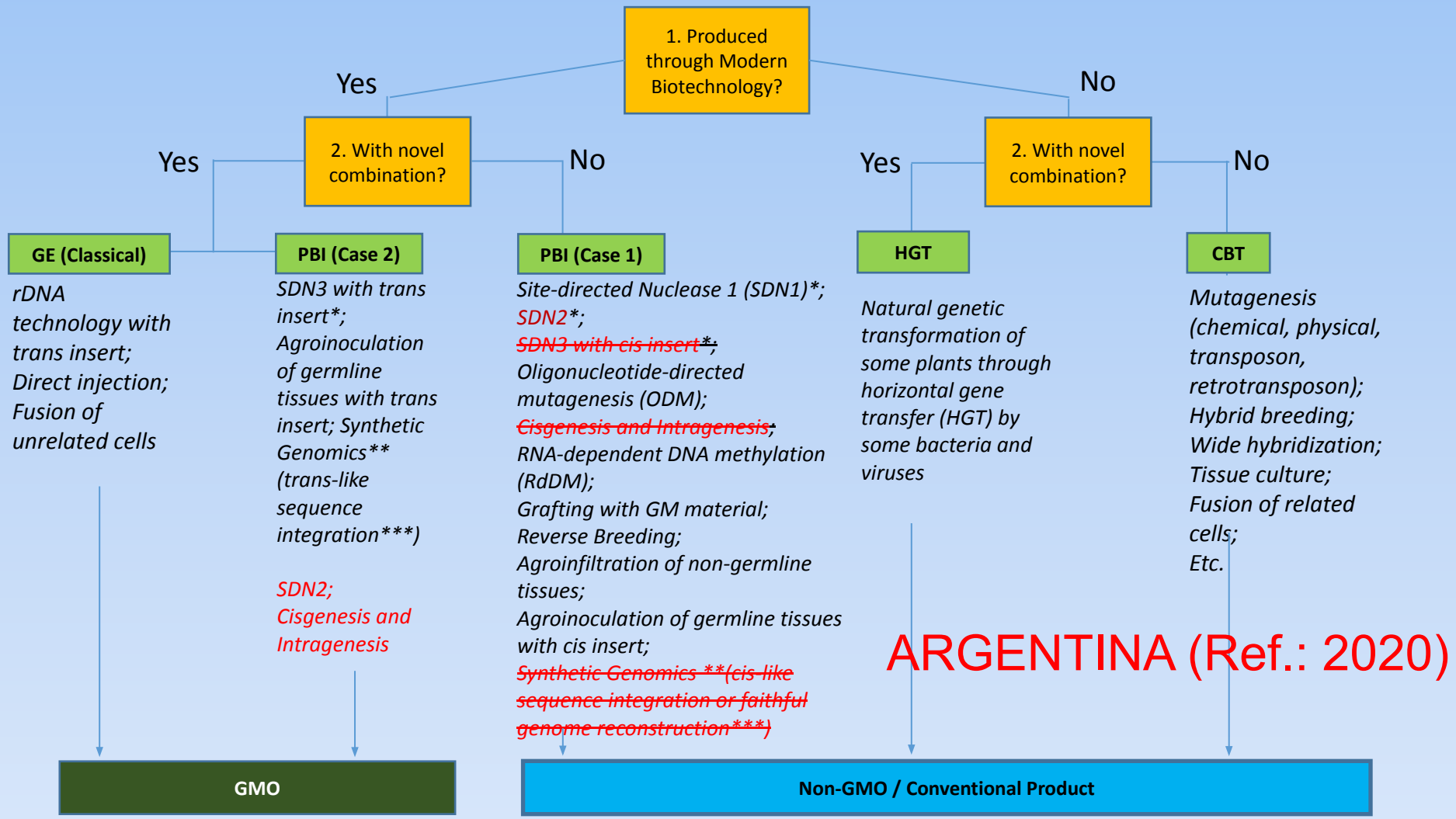

ARGENTINA (Ref.: 2020)

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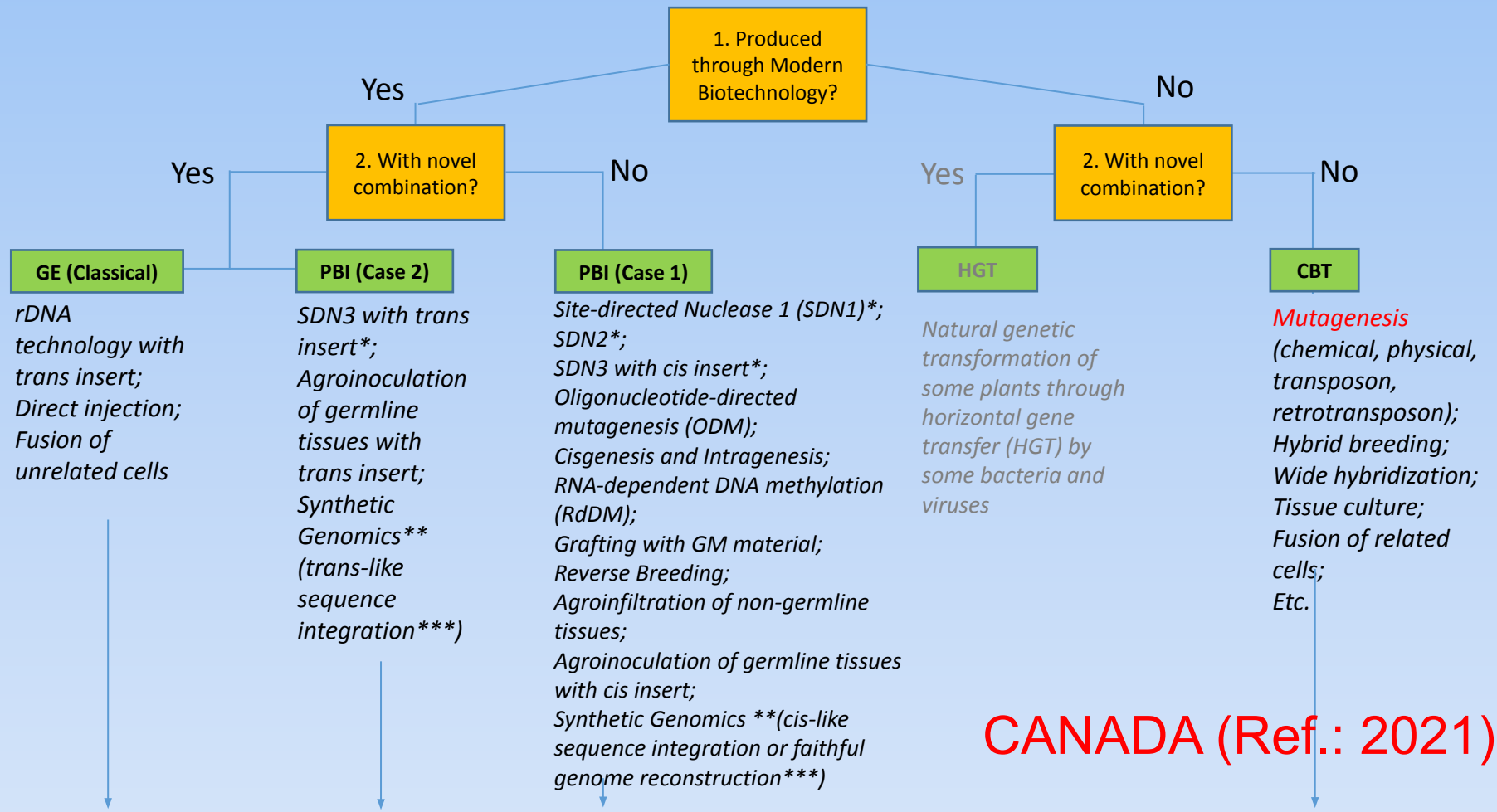

CANADA (Ref.: 2021)

Yes (PNT or GMO) or No (non-GMO)

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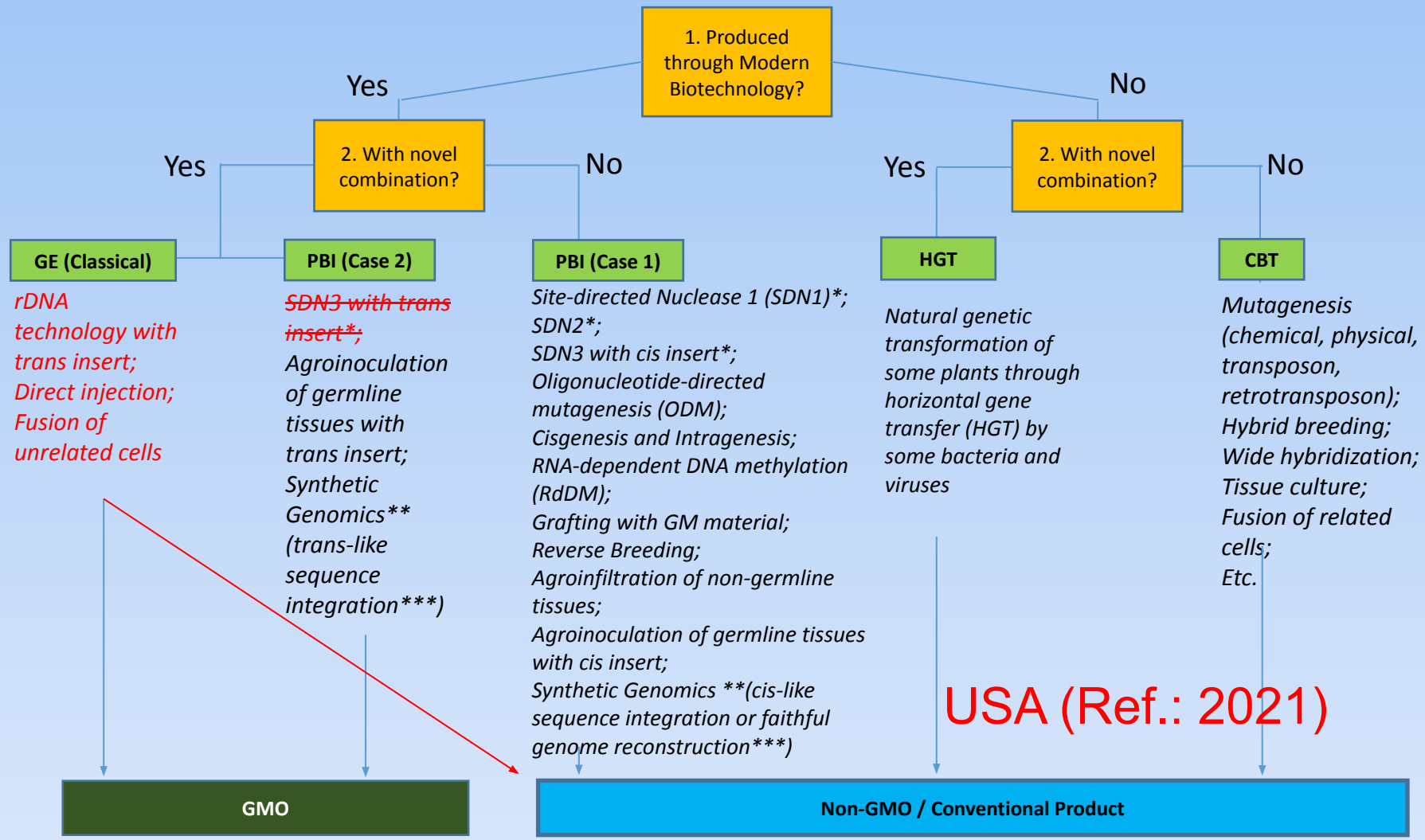

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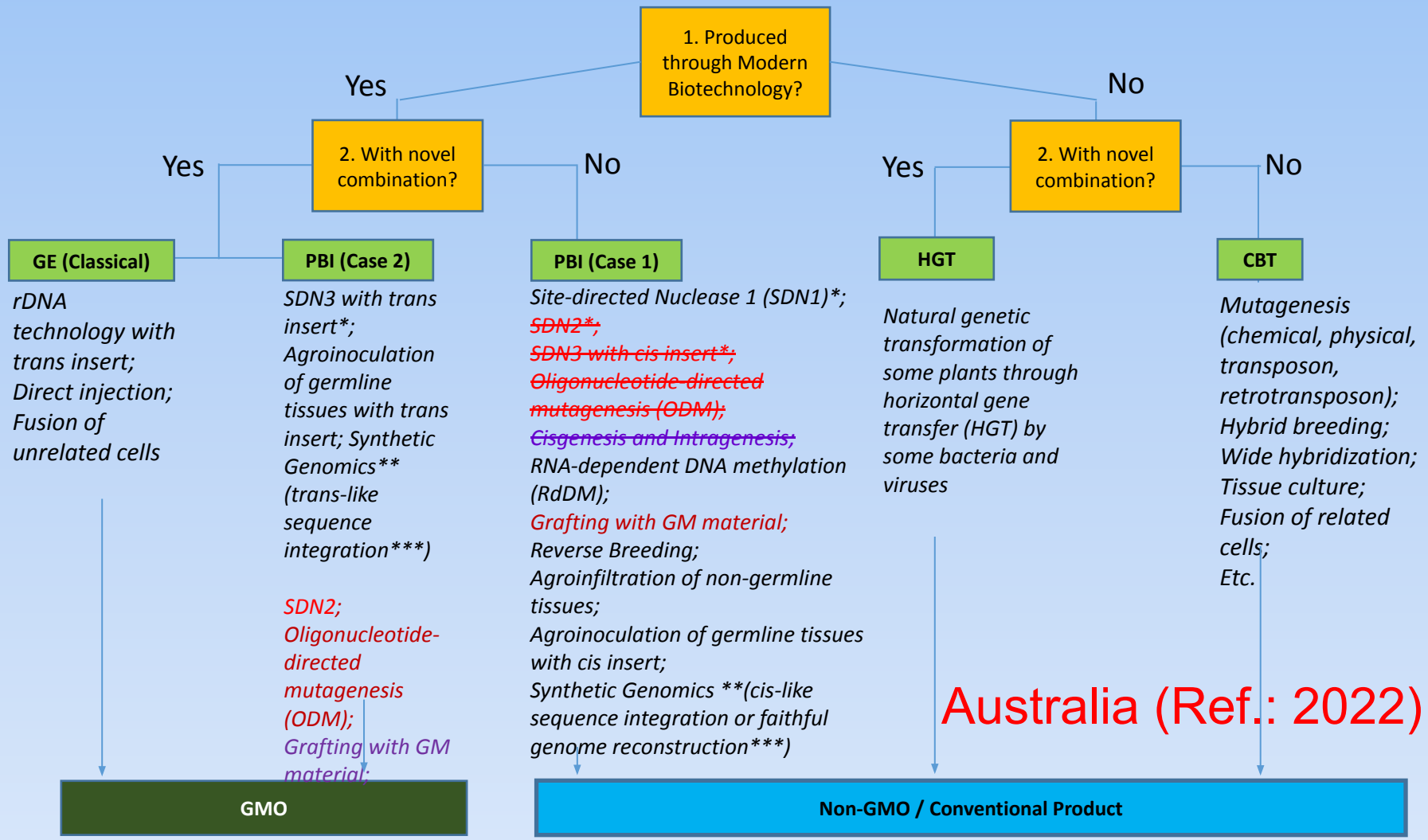

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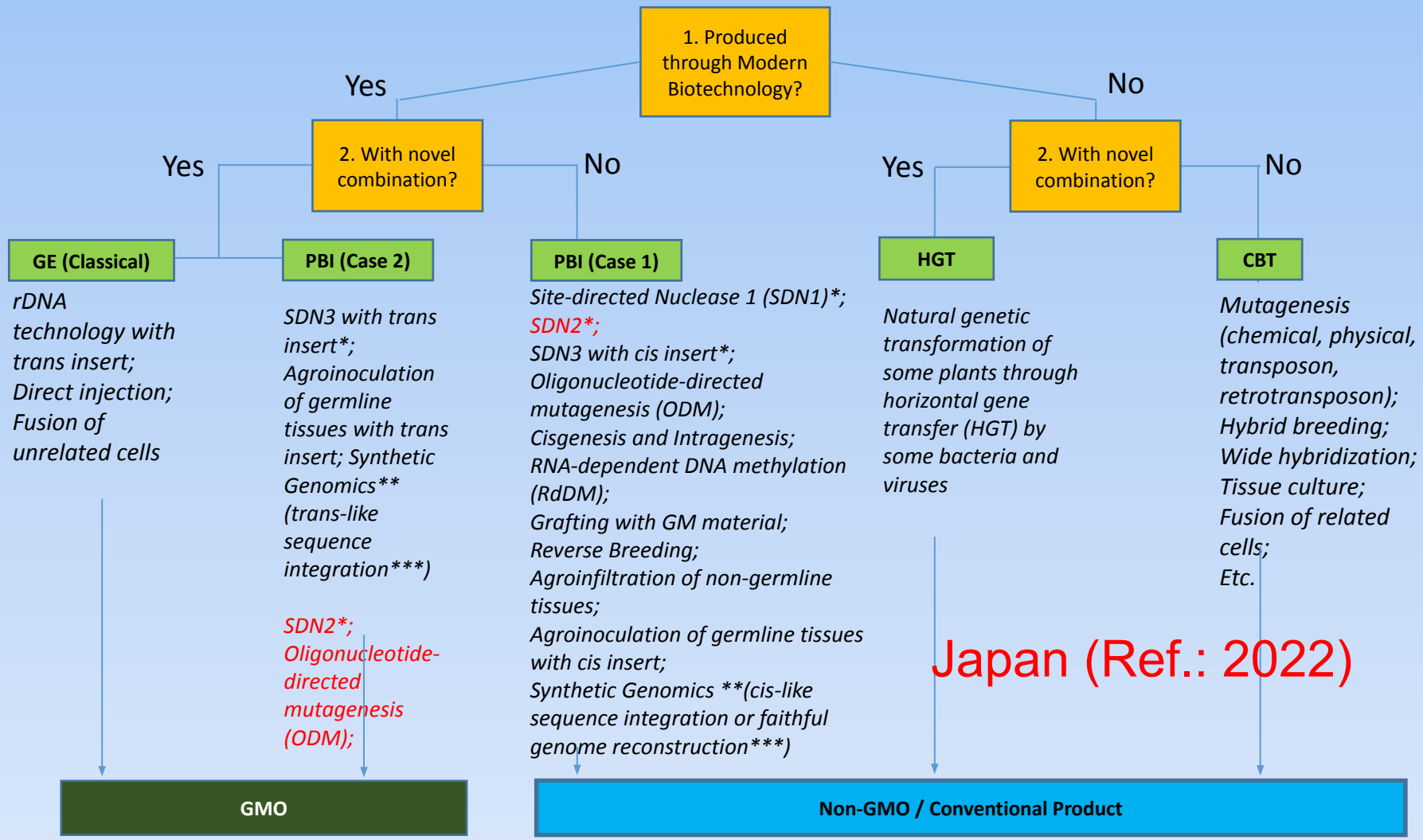

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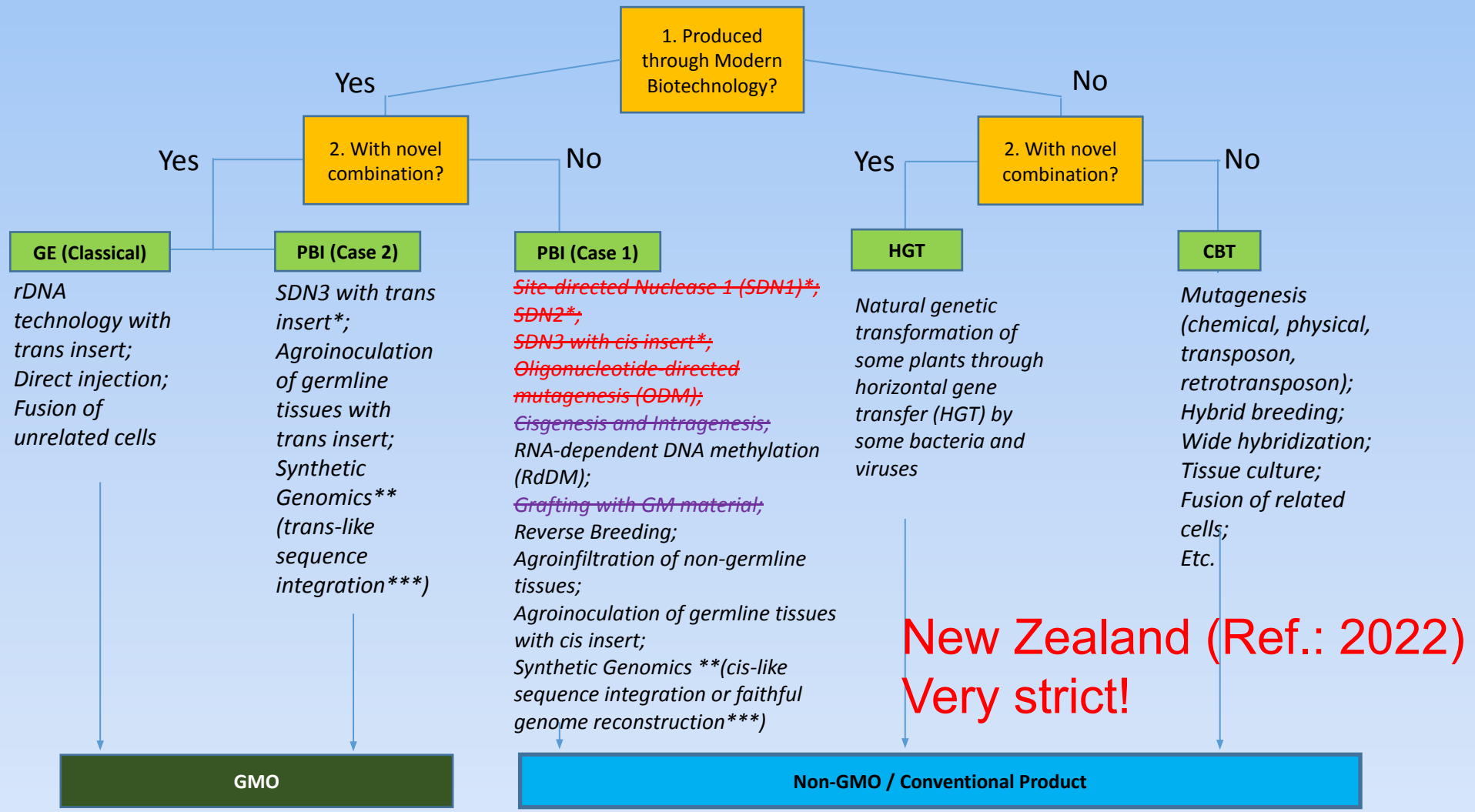

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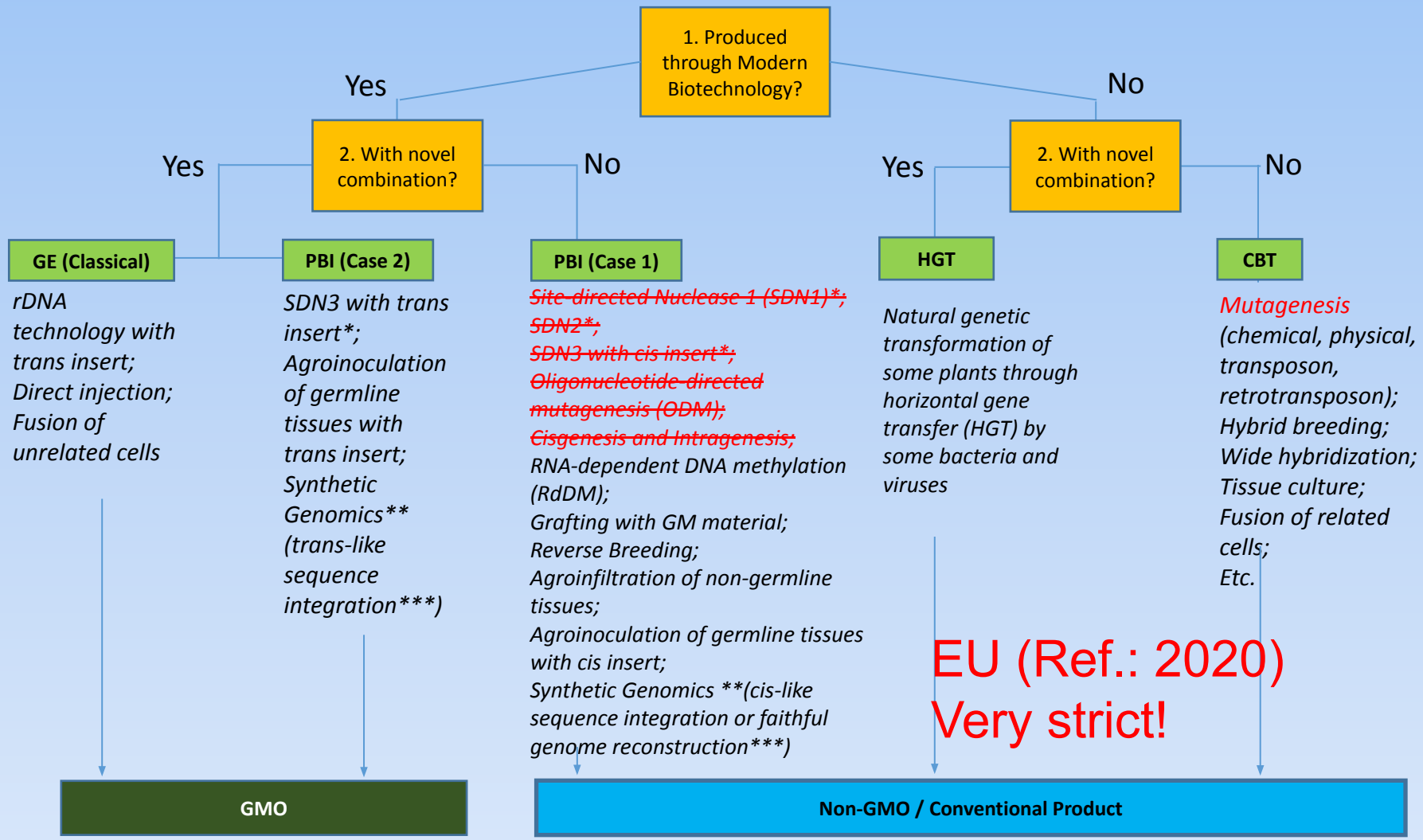

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Quality Rice. Quality Life.

Key Take-aways

- There are different new technologies available to the modern-day breeders and one of them is Plant Breeding Innovations (PBIs)
- Genome editing is the most popular among the PBIs
- Enabling policies are important for new technologies to have an impact on agriculture
- There are different perspectives globally on how PBIs are assessed (EU and New Zealand are very strict!).

Quality Rice. Quality Life.

Thank you!

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Some New Technologies for Plant Breeding

1. Advanced genome/transcriptome sequencing tools (e.g., NGS systems like Illumina, Pacbio, Nanopore)
2. Advanced data analytical tools (e.g., bioinformatics, AI)
3. Advanced DNA synthesis technologies (up to genome level)
4. Plant Breeding Innovations including Precision Breeding

Crops with Whole Genome Sequence information

Name	Details
Rice	Rice, Os-Nipponbare-Reference-IRGSP-1.0,
Corn	Corn, Zm-F7-REFERENCE-TUM-1.0, Zea mays subsp. mays (maize)
Coconut	coconut, UPLB_dcnu_1.0, Cocos nucifera (coconut palm)
Coconut	coconut, ASM812446v1
Mango	Mango, NCBI genome data viewer
Mango	Mango, CATAS_Mindica_2.1, Mangifera indica (mango), Cultivar: Alphonso
Garlic	GARLIC ; Allium sativum isolate Ershuizao, whole genome shotgun sequencing proje
Onion	ONION; allium cepa DHCU066619 genome sequence
Eggplant	Egplant; SME_r2.5.1
Tomato	Tomato, SL3.0, Solanum lycopersicum (tomato), Cultivar: Heinz 1706
Cotton	Cotton, Gossypium hirsutum (cotton), (Mexican cotton)
Rubber	Rubber tree, ASM165405v1, Hevea brasiliensis (rubber tree), Cultivar: reyan7-33-97
Cassava	Cassava, M.esculenta_v8, Manihot esculenta (cassava)
Cassava	Cassava, hifiasm152_l3.hic.hap2.p_ctg, Manihot esculenta (cassava), Cultivar: African
Sweet potato	Sweet potato, ipoBat4,Ipomoea batatas (sweet potato), Cultivar: Taizhong6
Potato	Potato, SolTub_3.0, Solanum tuberosum (potato), Cultivar: DM 1-3 516 R44
Yam	Yam, Dalata_v2, Dioscorea alata (monocots), Cultivar: TDa95/00328, (Purple Yam or Ube)
Cacao	Cacao, Criollo_cocoa_genome_V2, Theobroma cacao (cacao), Cultivar: B97-61/B2
Papaya	Papaya, Papaya1.0, Carica papaya (papaya), Cultivar: SunUp
Banana	Banana, ASM31385v2, Musa acuminata subsp. malaccensis (wild Malaysian banana), Strain: Doubled-haploid Pahang (DH-Pahang)
Abaca	upcoming