Plant Biotechnology

Bayer Russia Plant Biotechnology Conference

July 2023 – week 2

Bayer Russia Plant Biotechnology Conference:

Day 6	**Plant Care in CE & Intro to Molecular Assays**
Day 7	**Molecular Assays & Gene Editing Technology**
Day 8	**Molecular Assays & Model Systems**
Day 9	**Protoplast Systems**

Model Systems

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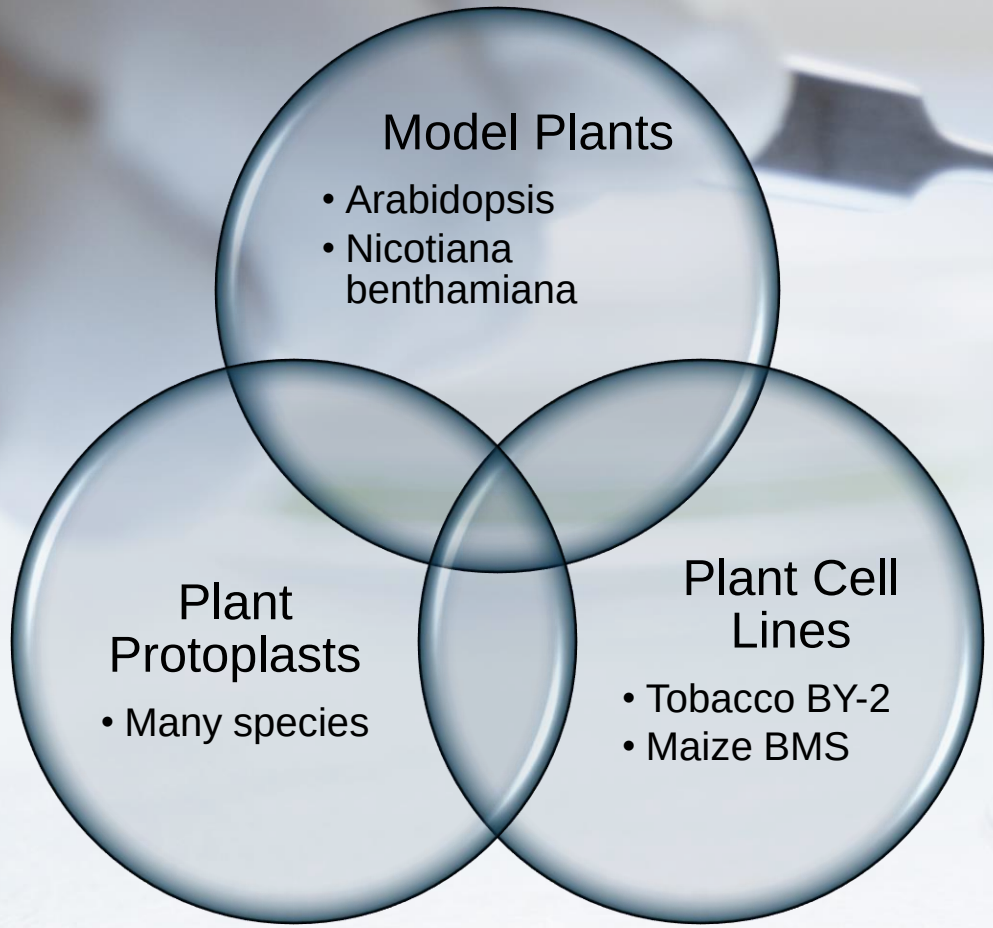
Model Systems

Model systems are widely used in plant molecular biology research and have been instrumental in advancing our understanding of plant biology. Speed, Cost, Flexibility, Genomic resources and Relevance to a biological question are key to selecting a Model System.



Model Systems

- ***Organisms or cellular systems that have been used to study & understand biological processes.***
- Extensive tools and genomic resources are available for research

	Genome size (Mb)	Life cycle
Arabidopsis	135	2 months
Nicotiana benthamiana	3,500	3 months
Medicago	~454–526	3-4 months
Micro-Tom	817	2–3 months
Rice	372	4 months
Corn	2,300	2-4 months

Rice Genome Hub

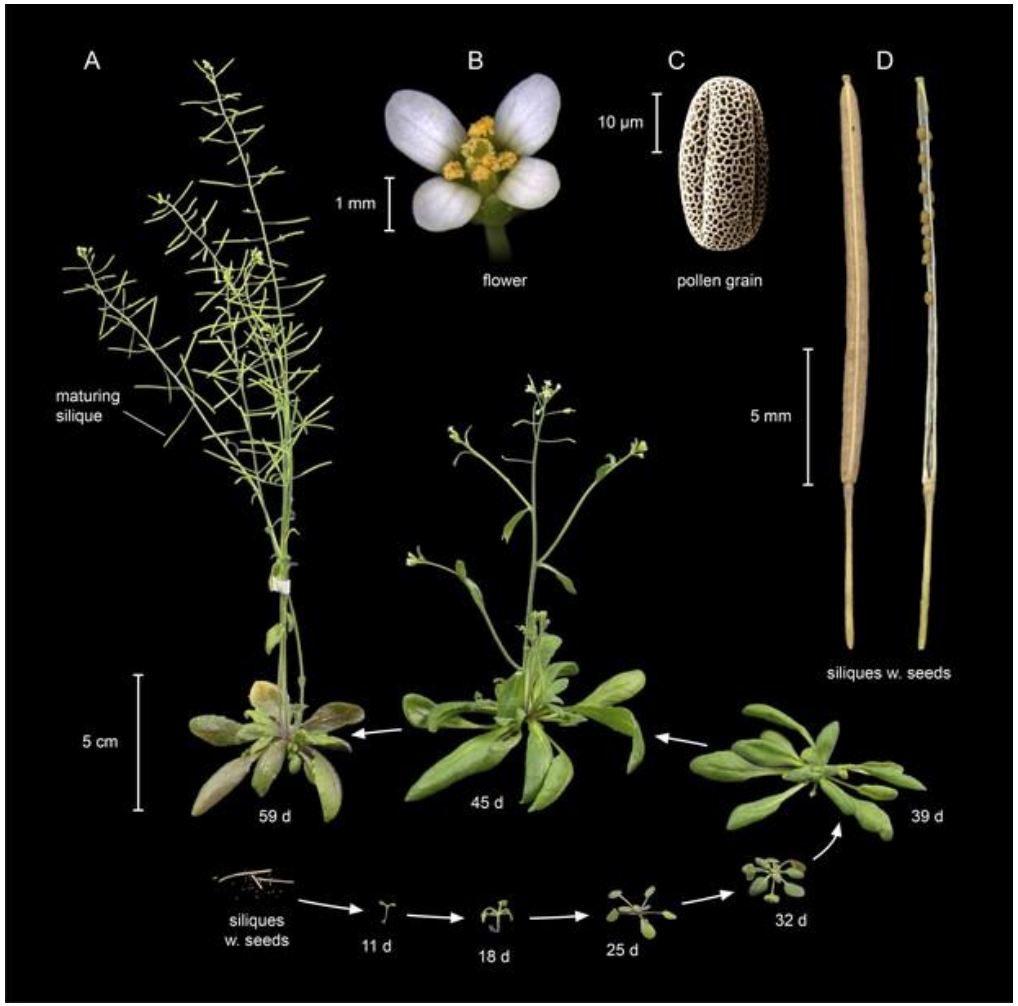
Arabidopsis

Advantages:

- **Short Lifecycle and plant size**(~60 days)
  - Large seed production per plant
- **Small genome extensively studied**
  - ~132 Mbp , ~38,000 loci, & >20,000 protein-coding genes

Disadvantages:

- **Dicot** (many food staple crops are monocots)
  - does not produce fruit
  - Missing genes relative to monocots
- The amount of information which can be extrapolated to fruit-bearing plants and cereals is limited.



https://doi.org/10.7554/eLife.06100.002

Additional Model Systems

- *Medicago truncatula*, nitrogen fixation (~454–526 Mb genome)

Nicotiana benthamiana

A close relative of tobacco, is a widely used model system with extensive genomics resources.

It is highly amenable to agroinfiltration and has a rapid growth rate, making it a popular choice for transient assays.

- ❖ Recombinant protein and chemical production
- ❖ Disease (virus and disease susceptible)
- ❖ Gene and promoter function
- ❖ Virus Induced Gene Silencing

Based on a comparison of the genome structures between N. benthamiana and N. tabacum, N. benthamiana was found to have more complex chromosomal rearrangements

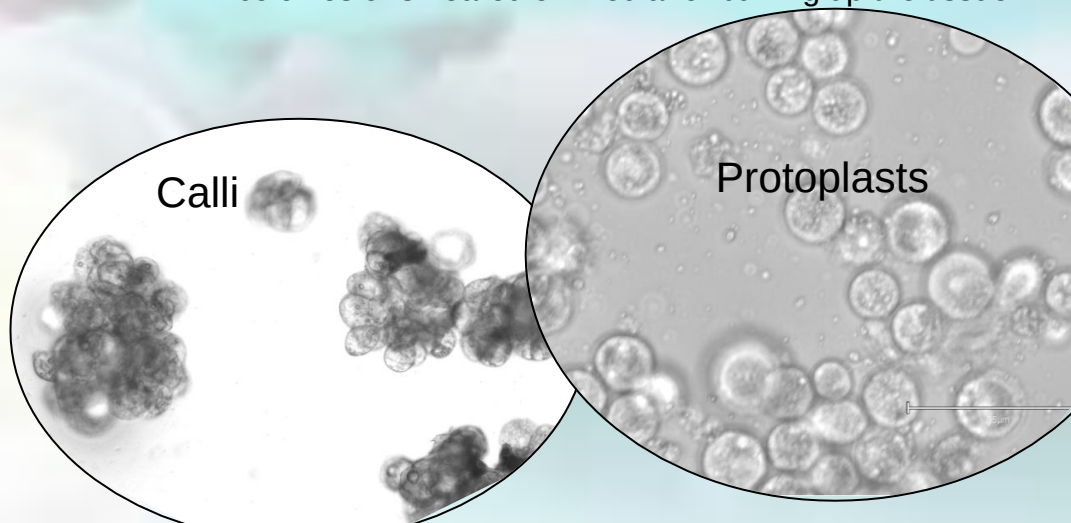
The genome assembly data, annotations and gene models are available at the NbenBase (https://nbenthamiana.jp)

Maize BMS (Black Mexican Sweet corn)

First isolated from the endosperm of immature corn kernels of the Black Mexican Sweet variety.

- **High growth rate**
- **Easy to culture and bulk up:** in simple, defined MS based-media
  - // Can be established as a suspension cell culture to use as a screening tool and to extract protoplasts, lysates, etc. for other studies
- **Corn model system:** A crop-specific, rapid expression model system to test genes and protein expression or small molecules.
- **Transformation:** callus can be transformed *via* biolistics or *Agrobacterium*
  - // Callus is non-regenerable (i.e., does not produce shoots/plantlets)

colonies or smeared on media for bulking up the tissue



Tobacco BY-2

established from a callus obtained from *Nicotiana tabacum* cv. BY-2 (cultivar Bright Yellow - 2)

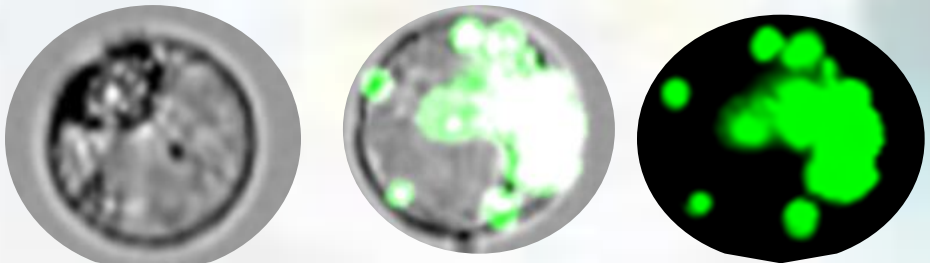
Easy to culture: in simple, defined media and are highly tolerant to osmotic stress and pH variations.

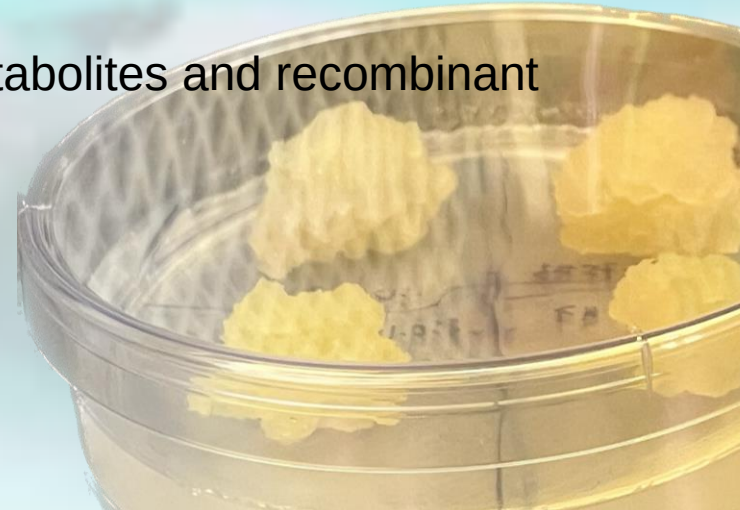
Homogeneous cell population

- High growth rate: 100-fold growth in a week, allows for rapid biomass accumulation and scale-up of culture.
- High transformation efficiency: used to study efficient gene expression and knockdown.

Conservation of metabolic pathways

- Suitable for large-scale production of secondary metabolites and recombinant proteins.
- Suitable for evolutionary and comparative biology.





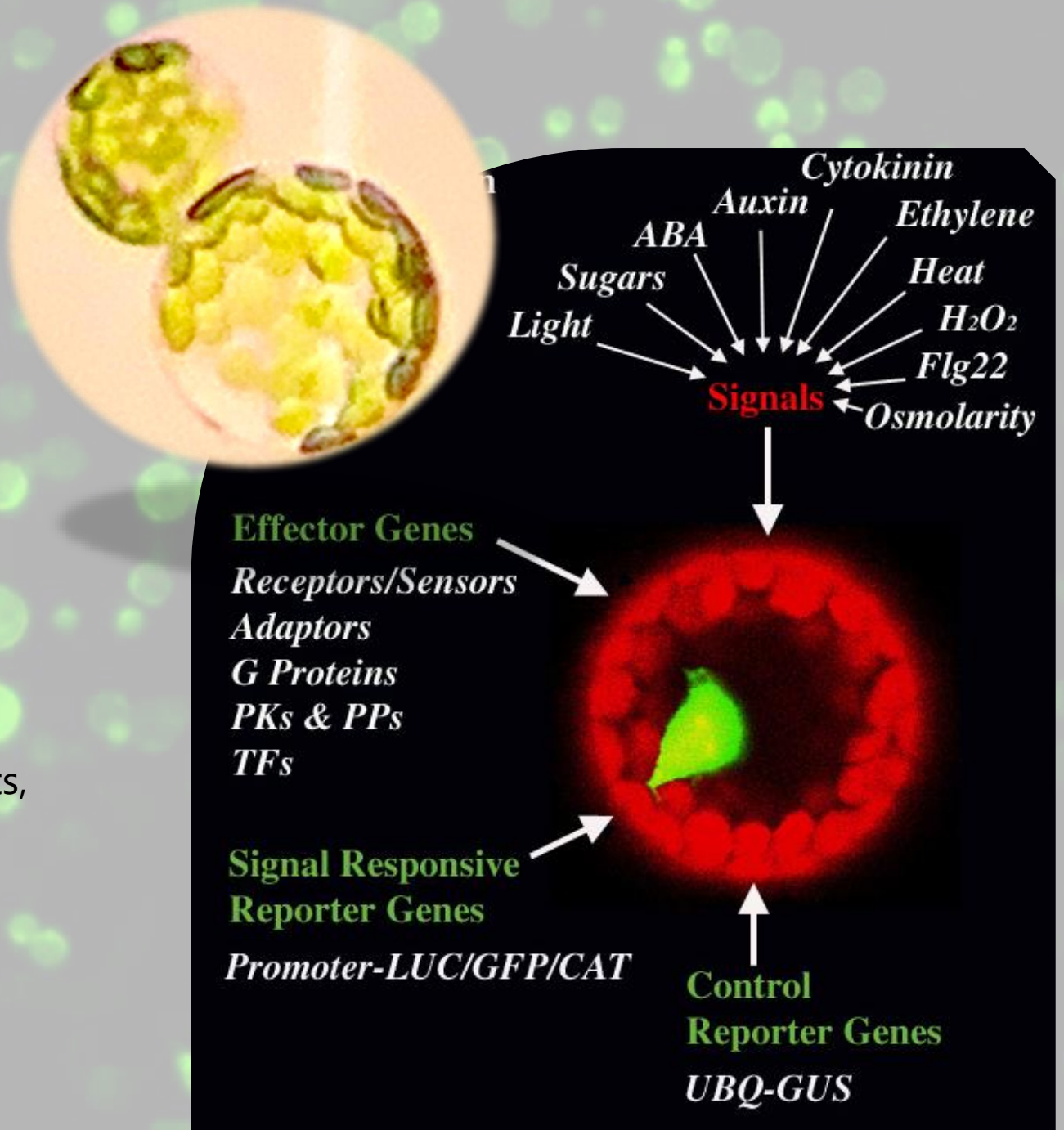
Plant Protoplasts

Primary cells – retain the identity of the isolated tissue

- **high transformation efficiency**
- **a homogeneous system**
- **flexibility in the cell type**
- **minimal background noise**
- **high reproducibility**

// Ideal for study of post-transcriptional events, signaling, protein dynamics and environmental factors.

// Lacks cell wall and cell-cell connections



Protoplast Transient Transformation Protocols Available

Arabidopsis thaliana (Asai et al., 2002; Boudsocq et al., 2004, 2010; Bethke et al., 2009; Li et al., 2019)

maize (Kovtun et al., 1998)

rice (Takai et al., 2007; Wang et al., 2014; Liu et al., 2018)

barley (Saur et al., 2019)

wheat (Hahn et al., 2020)

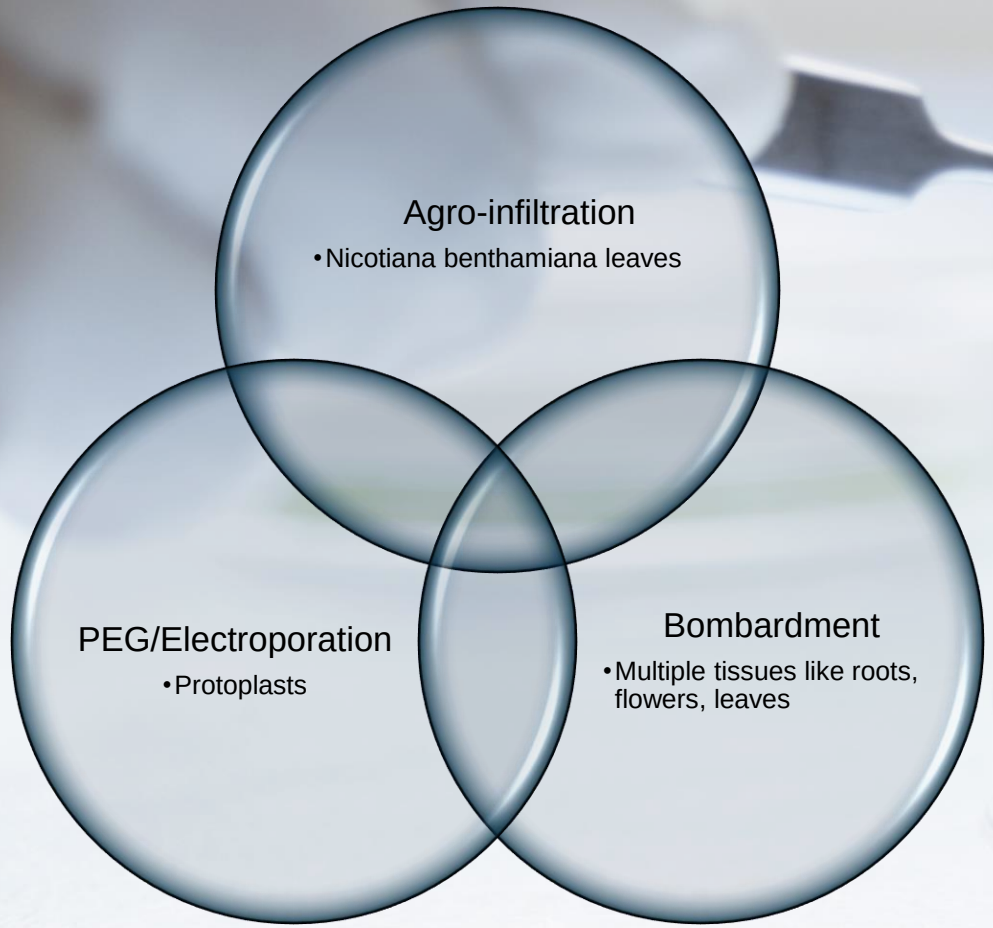
strawberry (Gou et al., 2020)

banana (Wu et al., 2020b)

rubber tree (Zhang et al., 2016)

Transient Assays

Several methods are used to transiently transform plant tissues or cells with the aim of assessing expression elements, genes, and molecular mechanisms of cell biology. Transient expression can range from minutes up to 3-5 days after transfection.



Agro-infiltration

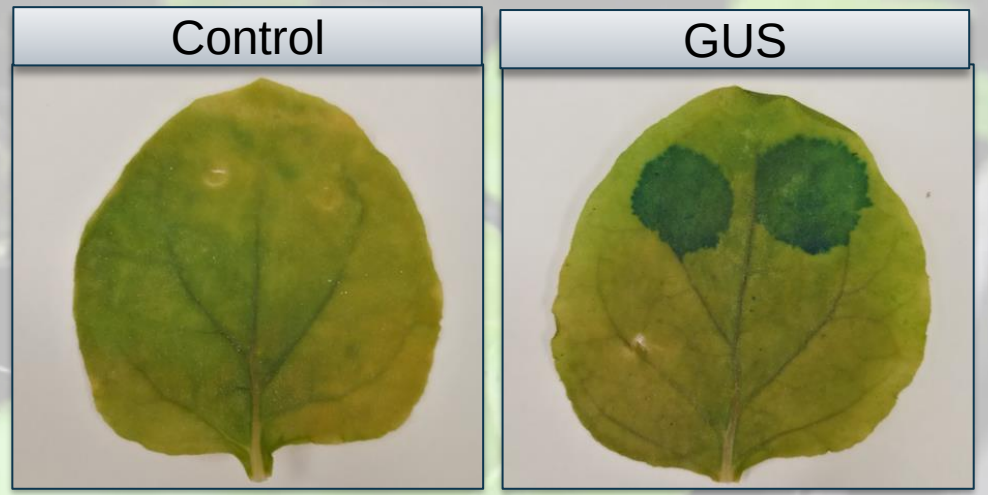
Underside of leaf is nicked, followed by infiltration of Agrobacterium without a needle.

Figure 1: Syringe agroinfiltration of *N. benthamiana* leaves with *Agrobacterium tumefaciens*. *A. tumefaciens* harboring the gene of interest was resuspended in infiltration buffer and loaded into a syringe without a needle. A nick was created with a needle on the backside of a 6-week old plant leaf (A). *Agrobacteria* were injected into the interstitial space of the leaf via the nick (B and C) [39].

Chen et al., Adv Tech Biol Med 2013, 1:1
DOI: 10.4172/2379-1764.1000103

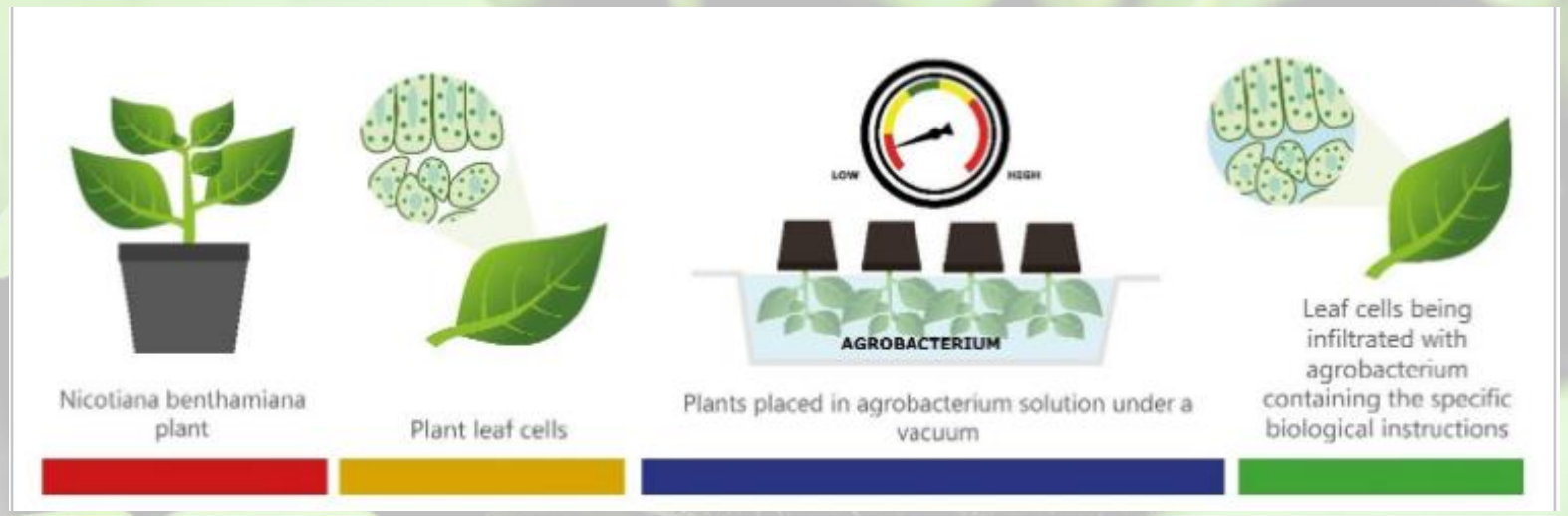
Agro-infiltration

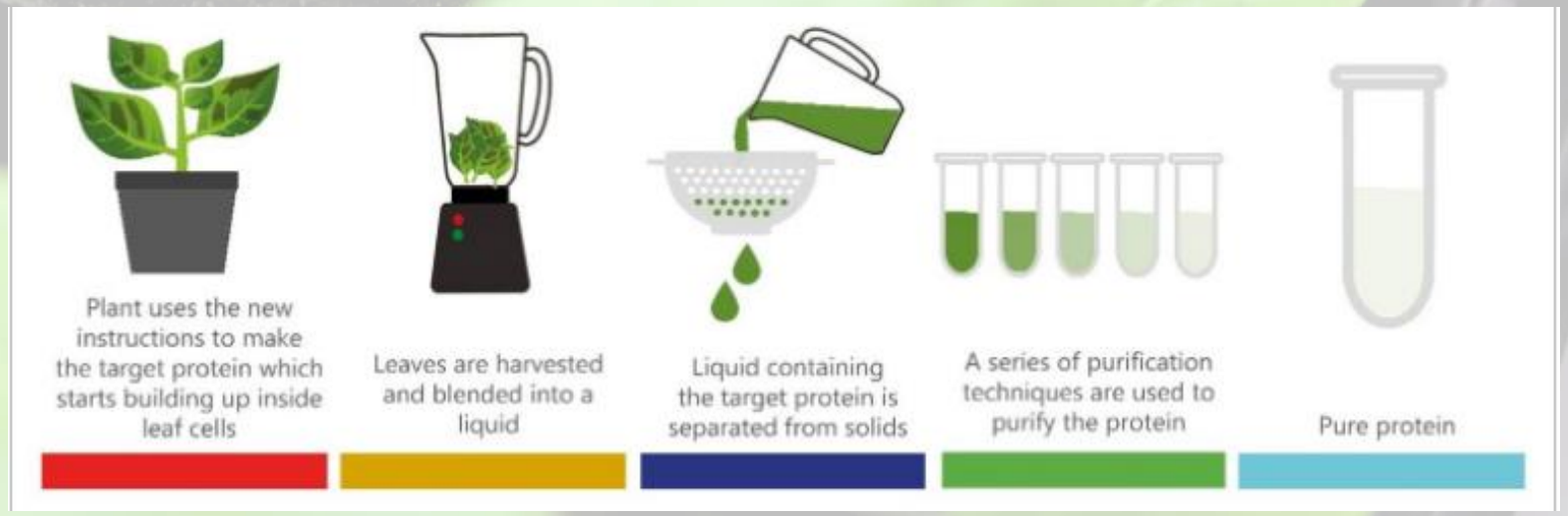
Expression is observed 2-7+ days after infiltration



Agro-infiltration

Scaled-up version can be used to produce medicines or vaccines in 4 months (i.e. Virus free polio vaccine)





https://gott.blog.gov.uk/2020/11/23/leaf-expression-systems-how-harnessing-the-power-of-plants-is-changing-the-future-of-our-medicines/
https://www.leafexpressionsystems.com/what-we-do

Agro-infiltration

Advantages

High efficiency of gene delivery
Agroinfiltration can result in high levels of expression of the introduced genes, making it useful for functional analysis of genes and promoters.

No need for plant transformation
Agroinfiltration transient assays do not require the production of transgenic plants, which can be time-consuming and expensive.

Easy to perform:
Agroinfiltration is a relatively simple and quick technique that can be performed without specialized equipment.

Good reproducibility:
Agroinfiltration transient assays can be easily replicated, allowing for good reproducibility of experimental results.

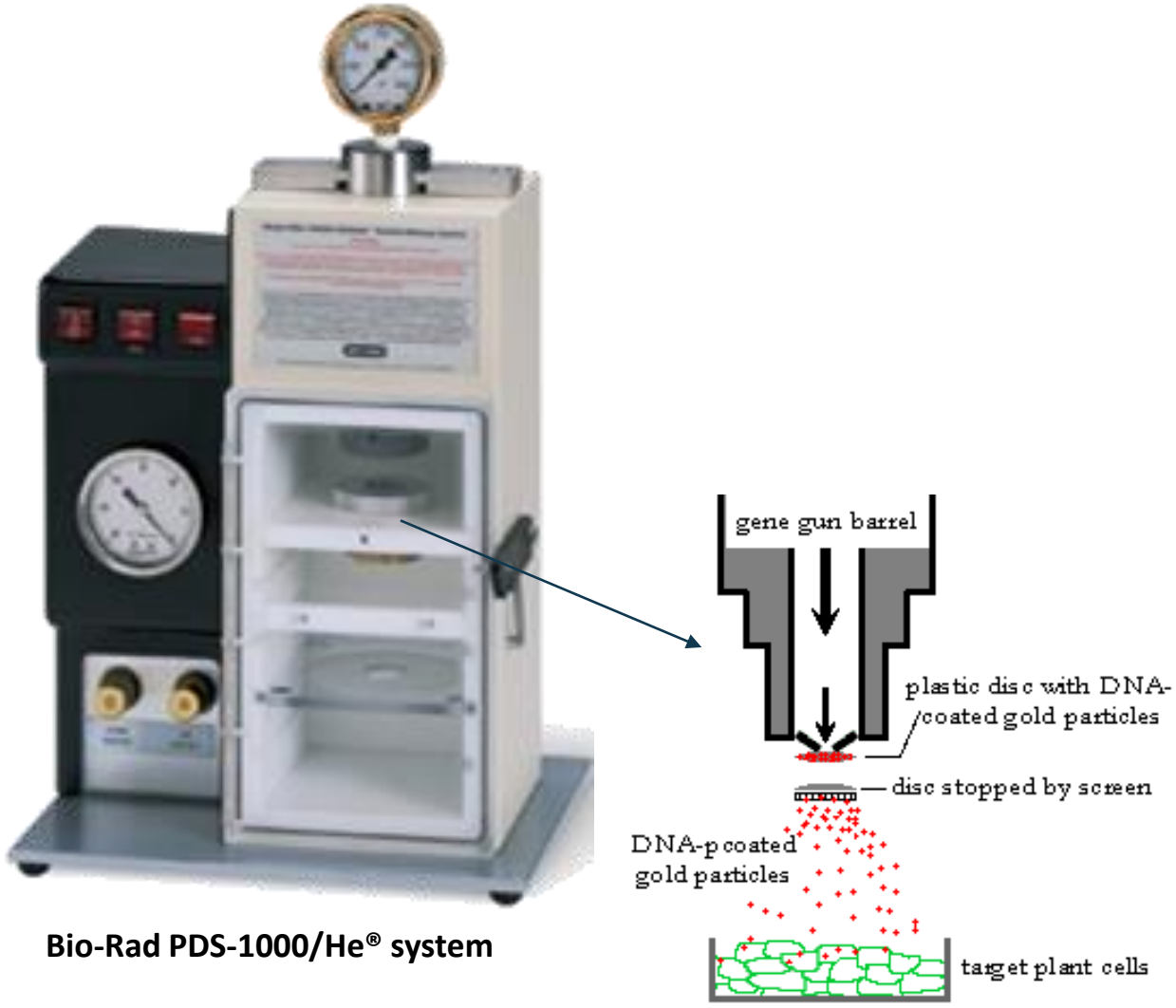
Limitations

Host range limitations:
Agroinfiltration is typically limited to a small number of plant species and may not be effective in all plant systems.

Limited duration of expression

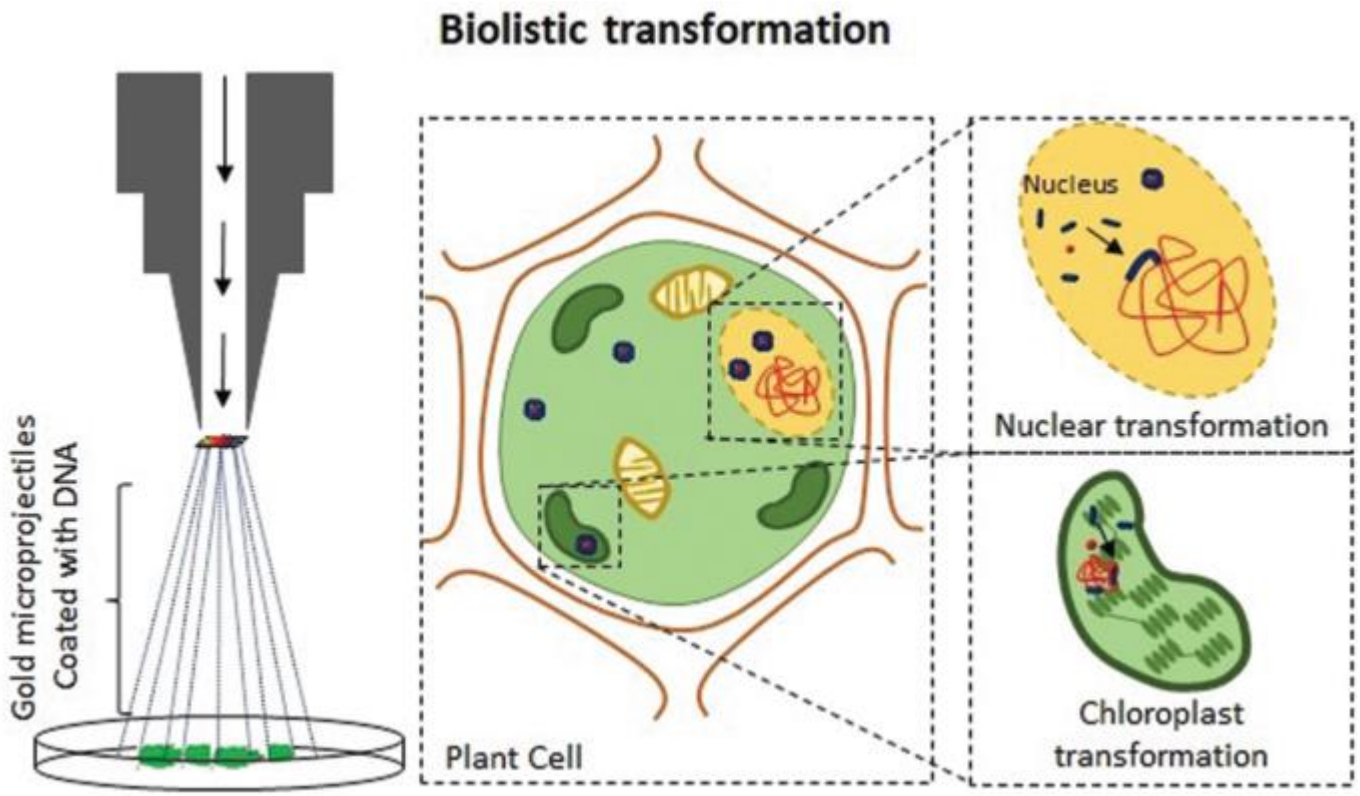
Brief intro on application of biolistic delivery system in plant biology

- Invented by researchers at Cornell University and DuPont in 1983-1986 (particle gun, gene gun, bombardment, biolistics)
- An alternative to agrobacterium-based delivery method
- Can be used for both transient and stable transformation
- Flexible for genotypes and tissue types



Bio-Rad PDS-1000/He® system

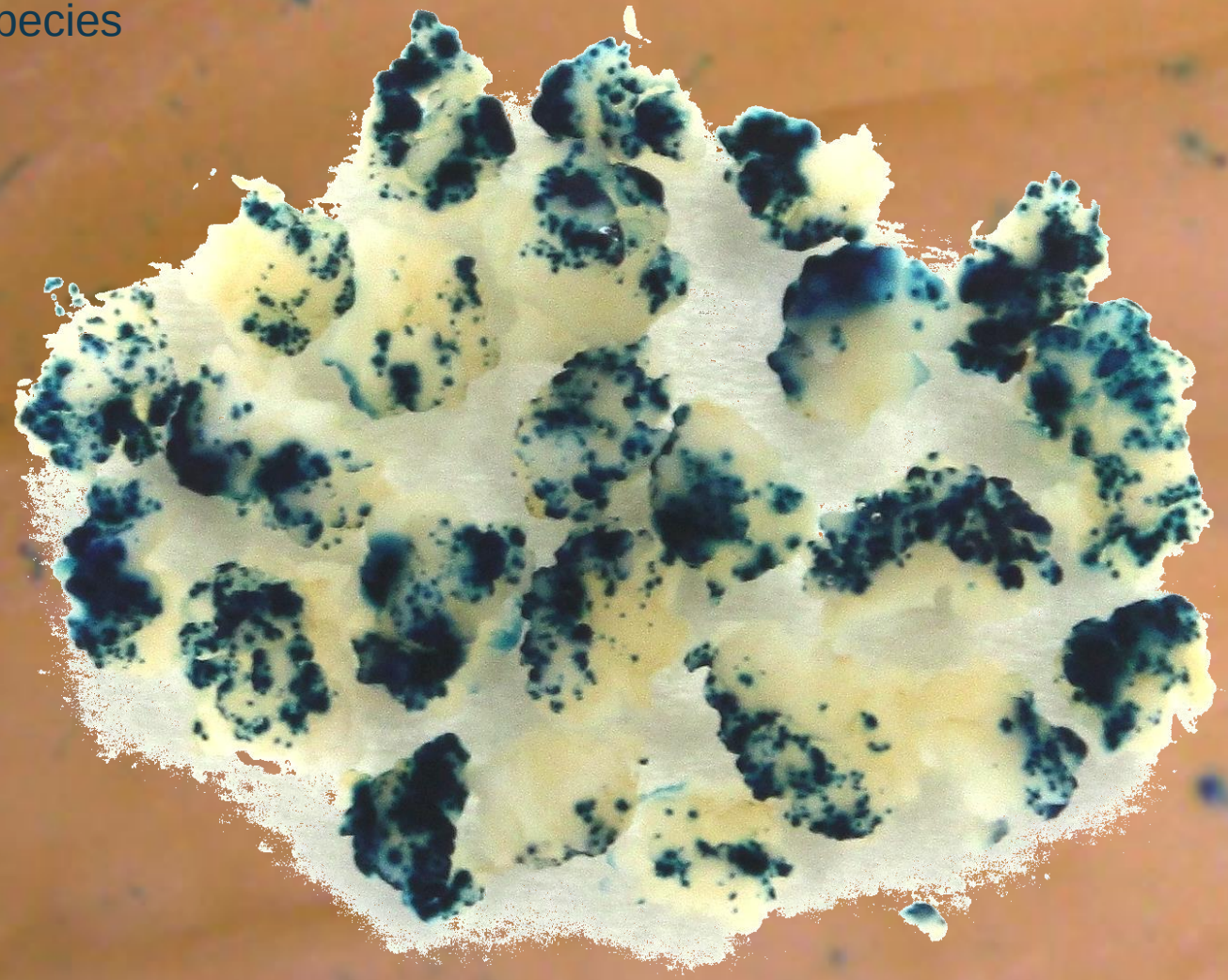
Bombardment

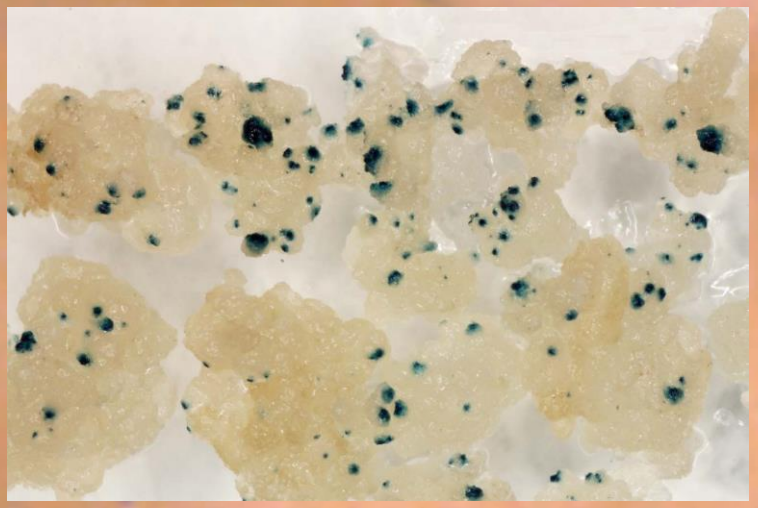


https://link.springer.com/protocol/10.1007/978-1-0716-0356-7_3

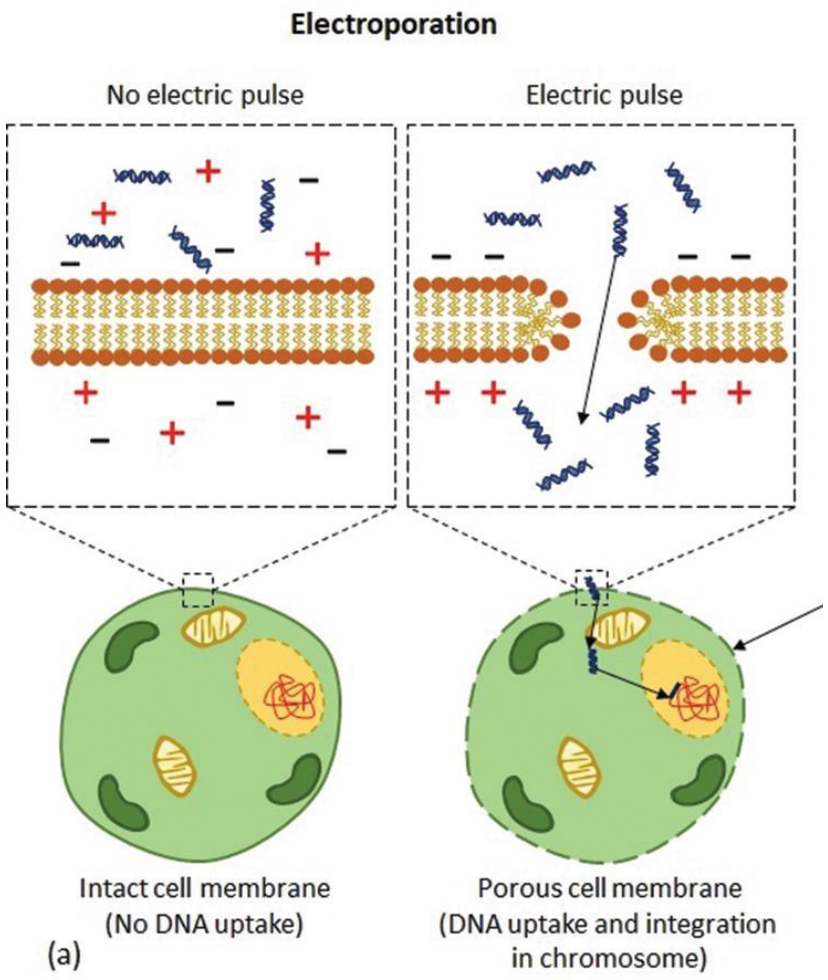
Biolistic Transformation

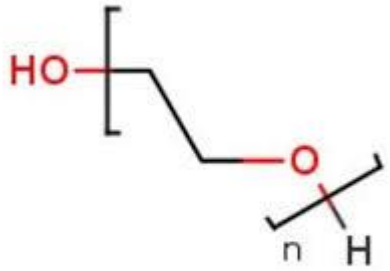
Enables transformation of a variety of tissues. Efficiency varies and can be noisy, depending on tissue and species





PEG/Electroporation Transformation in Plant Protoplasts





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Thank you!

Any questions?