

Reporter gene

Programme: B.Sc (H) Botany

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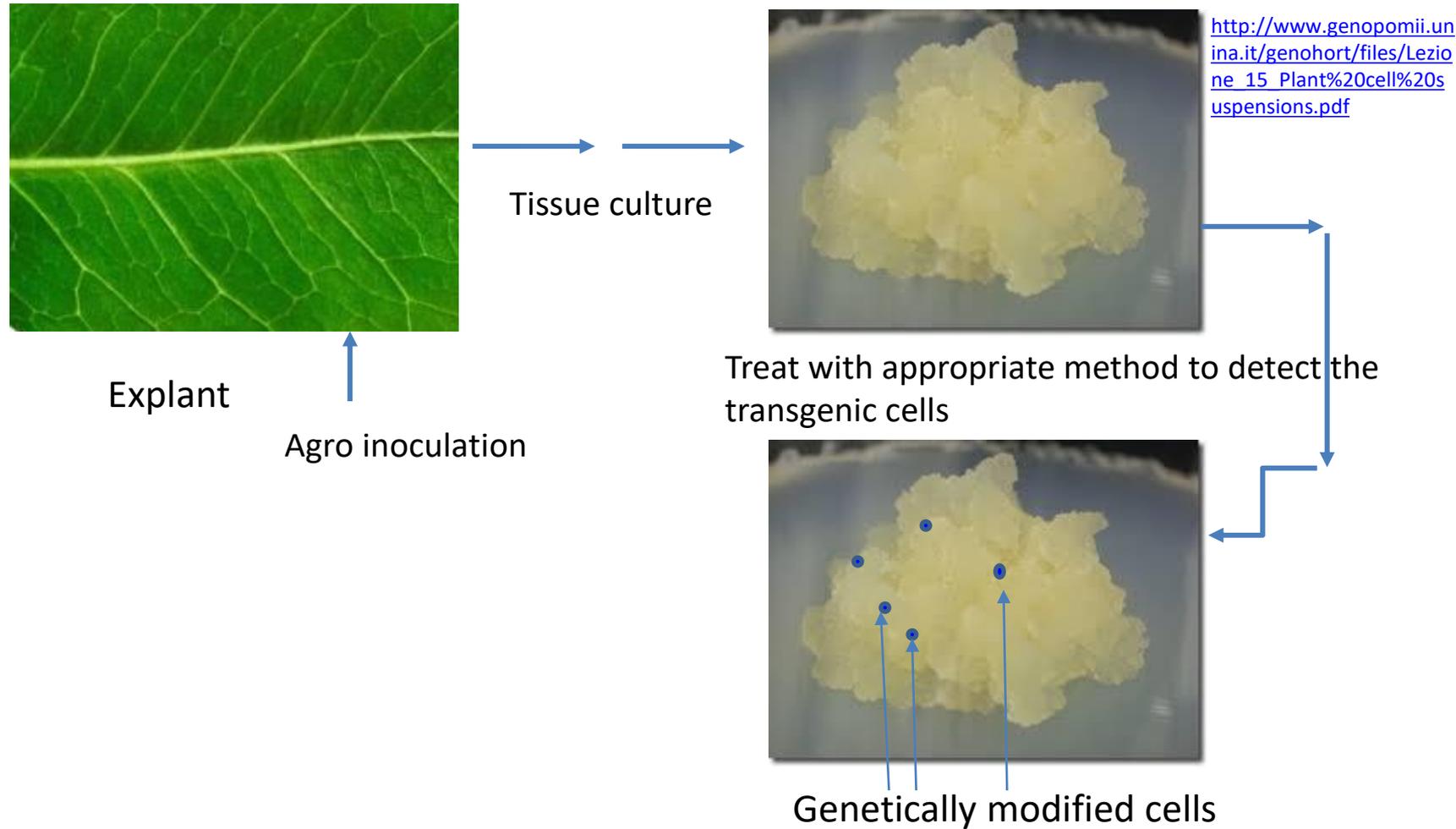
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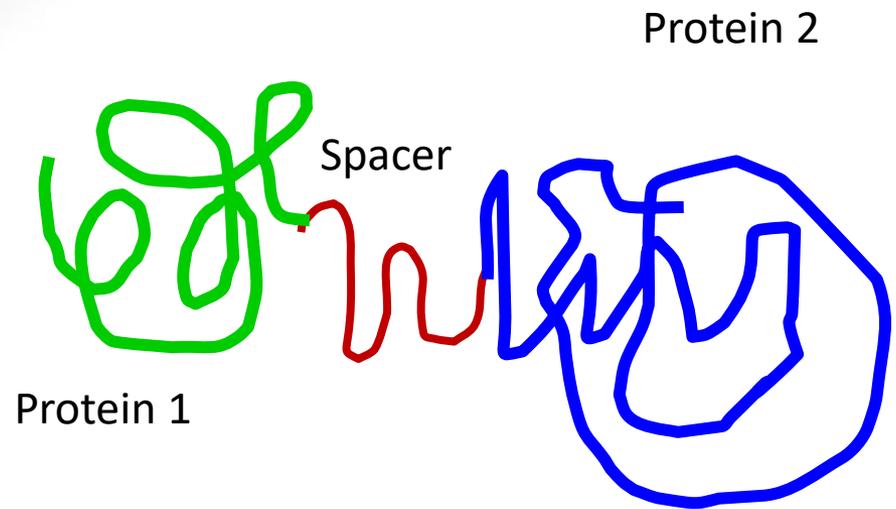
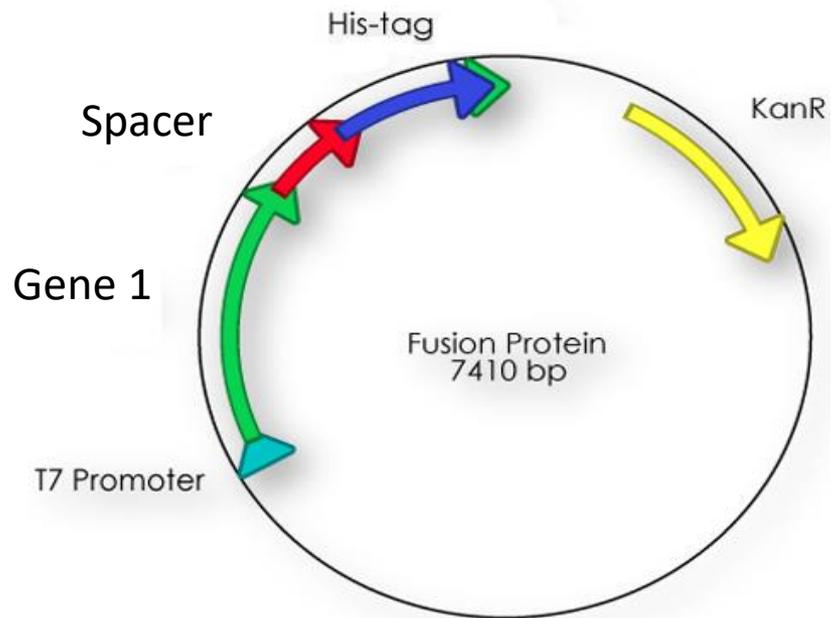
Selection of a transgene

- The use of a marker gene in a transformation process aims to select transformed cells that is expressing the gene of Interest.
- These selectable marker genes are often known as Reporter gene
- Such genes are called reporters because the characteristics they confer on organisms expressing them are easily identified and measured.

Marker genes for selection enable the identification and selection of genetically modified cells.



- To introduce a reporter gene into an organism, scientists place the reporter gene and the gene of interest under the control of same promoter in the same DNA construct .
- This typically involves **removing** the **stop codon** from a cDNA sequence coding for the first protein, then appending the cDNA sequence of the second protein in frame through ligation .



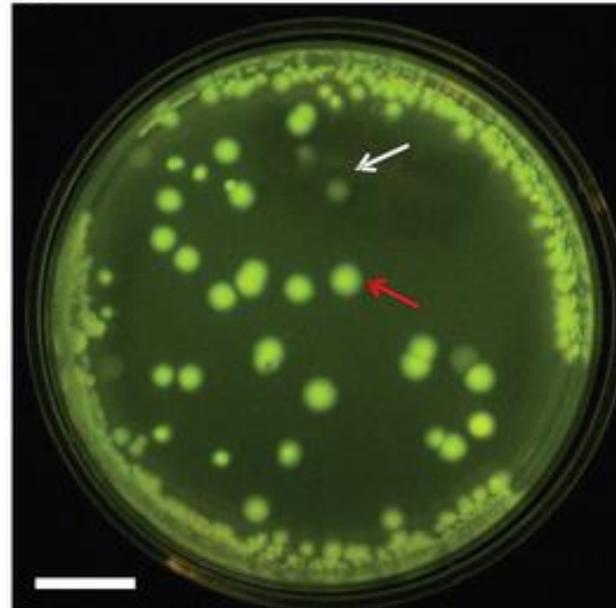
- Here often a linker (or "spacer") peptides are also added, which make it more likely that the proteins fold independently and behave as independent proteins
- It is important to use a reporter gene that is not natively expressed in the cell or organism under study, since the expression of the reporter is being used as a marker for successful uptake of the gene of interest.

- Commonly used reporter genes that induce visually identifiable characteristics usually involve **fluorescent** and **luminescent** protein or some compound that gives a colored reaction

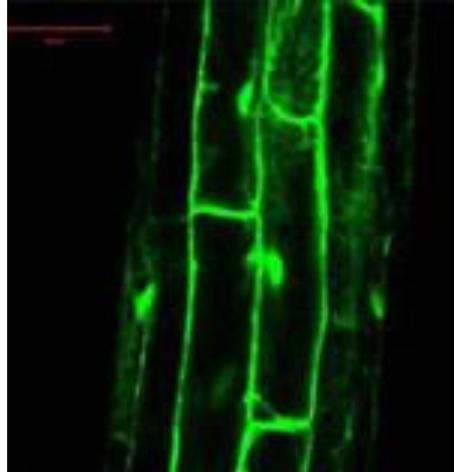
Green fluorescent protein

- Examples include the gene that encodes jellyfish **green fluorescent protein (GFP)**, which causes cells that express it to glow green under blue light.
- Colonies if expressing can be detected under UV light most of the time

White arrow shows colony with negative expression
Red arrow indicates expression of reporter and desired protein



- Green fluorescent protein (GFP) fluorescence can be observed via confocal laser scanning microscopy .



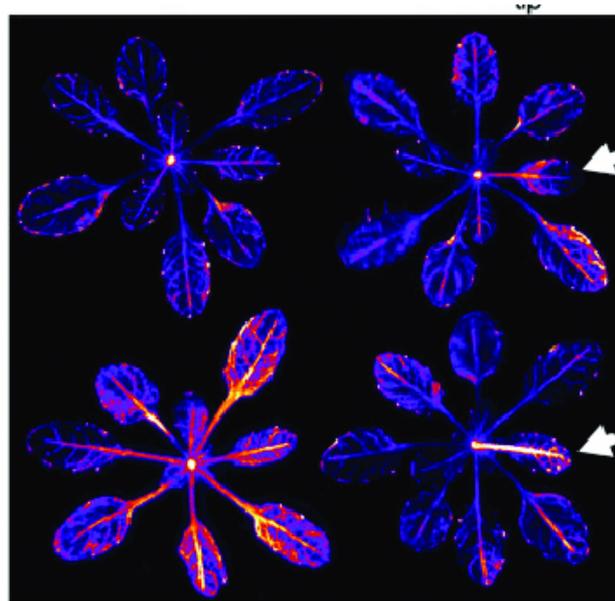
Subcellular localization of GFP in transgenic plants.

•DOI: [10.1104/pp.16.01106](https://doi.org/10.1104/pp.16.01106)

Luminescent proteins

- The gene for enzyme luciferase, which catalyzes a reaction with luciferin to produce light, is often used as reporter .
- LUC plants parts are exposed to the light for 7 h. Whole shoots or single leaves are cut and evenly sprayed with 2 mM D-luciferin potassium salt in 0.1% (vol/vol) Triton X-100.

- Luciferase luminescence is imaged in a imager.
- Relative luciferase intensity in the petiole is analyzed by measuring the mean pixel intensity of the plant parts.

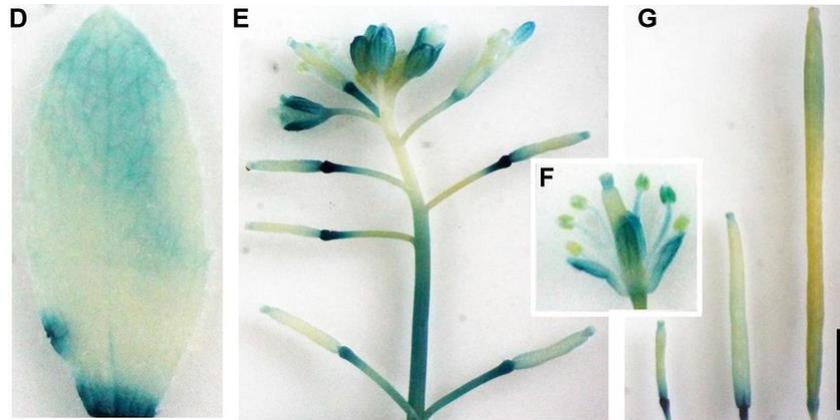


Luciferase luminescence of the LUC reporter on the abaxial side

GUS assay

- The GUS gene has been commonly used in plants but luciferase and GFP are becoming more common
- The gene codes for the β -glucuronidase enzyme (GUS) and was isolated from *Escherichia coli*. This gene is widely used as a reporter gene in transgenic plants.
- The most common substrate for GUS histochemical staining is **5-bromo-4-chloro-3-indolyl glucuronide** (X-Gluc): the product of the reaction is in this case a clear blue color.

- When the gene successfully expresses in the cells will produce the enzyme. That in turn will react to its substrate in the medium and Turn blue



GUS reporter gene in Arabidopsis transgenic plants

- Typical reporter genes are:

Gene	Product	Assay
<i>lacZ</i>	beta-galactosidase	indicator plates, colorimetric enzyme assay of cell extracts, selection for Lac ⁺
CAT	chloramphenicol acetyltransferase	enzyme assay or ELISA for gene product, selection for chloramphenicol resistance
<i>gus</i>	beta-glucuronidase	indicator plates, colorimetric enzyme assay of cell extracts -- used a lot in plants
GFP	green fluorescent protein	fluorescence of colonies, fluorescence of cells or subcellular compartments, imaging live cells

- Reporter genes offer a big advantage, because one doesn't need a separate assay for each regulatory region being studied.
- Also, for many reporter genes simple indicator plate assays have been developed that allow one to determine levels of expression by the color of a bacterial or yeast colony, and perhaps to isolate mutants based on changes in the color.

- For instance, a bacterial strain making substantial amounts of beta-galactosidase will form a blue colony on a plate with X-gal; this allows one to identify cells with different levels of beta-galactosidase, and to use X-gal plates for any regulatory region driving expression of beta-galactosidase.

Resources

- <https://doi.org/10.1590/S1677-04202002000100001>
- [s://en.wikipedia.org/wiki/Reporter_gene](https://en.wikipedia.org/wiki/Reporter_gene)