Genetic Engineering of Plants: Methodology

- Plant transformation with the Ti plasmid of *Agrobacterium tumefaciens*
- Ti plasmid-derived vector systems
- Microprojectile bombardment
- Chloroplast engineering
- Use of reporter genes in transformed plant cells
- Manipulation of gene expression in plants
- Production of marker-free transgenic plants

Why genetically engineer plants?

- To improve agricultural, horticultural or ornamental value of a plant (much faster than conventional plant breeding)
- To serve as a living bioreactor for production of economically important proteins or metabolites
- To produce vaccines or antibodies for human health
- To provide a renewable source of energy
- To study the role of genes (and gene products) in plant growth and development

Table 10.1

Table 10.1 Some pharmaceutical proteins that have been produced in transgenic plants

Pharmaceutical protein	Plant(s)	Application(s)
α-Tricosanthin	Tobacco	HIV therapy
Allergen-specific T-cell epitope	Rice	Pollinosis
Angiotensin-1-converting enzyme	Tobacco, tomato	Hypertension
Cyanovirin-N	Tobacco	HIV micobicide
Glucocerebrosidase	Tobacco	Gaucher disease
Human α1-antitrypsin	Rice, tomato	Cystic fibrosis, liver disease, hemorrhage
Human apolipoprotein	Safflower	Plaque reduction
Human aprotinin	Corn	Trypsin inhibitor for transplantation surgery
Human enkephalins	Arabidopsis, canola	Antihyperanalgesic by opiate activity
Human epidermal growth factor	Tobacco	Wound repair, control of cell proliferation
Human erythropoietin	Tobacco	Anemia
Human granulocyte-macrophage colony-stimulating factor	Tobacco	Neutropenia
Human growth hormone	Tobacco	Dwarfism, wound healing
Human hemoglobin	Tobacco	Blood substitute
Human hirudin	Canola, tobacco	Thrombin inhibitor, anticoagulant
Human homotrimeric collagen I	Tobacco	Collagen synthesis
Human insulin	Potato, *Arabidopsis*, safflower	Diabetes
Human α interferon	Rice, turnip	Hepatitis C and B
Human β interferon	Tobacco	Antiviral
Human interleukin-2 and interleukin-4	Tobacco	Immunotherapy
Human lactoferrin	Potato, rice	Antimicrobial, diarrhea
Human muscarinic cholinergic receptors	Tobacco	Central and peripheral nervous systems
Human placental alkaline phosphatase	Tobacco	Achonodroplasia or cretinism in children
Human protein C	Tobacco	Anticoagulant
Human serum albumin	Tobacco	Liver cirrhosis, burns, surgery
Human somatotropin	Tobacco	Growth hormone
Lipase	Corn	Cystic fibrosis

Table 10.2

Table 10.2 Examples of potentially therapeutic antibodies and antibody fragments that have been produced in plants

Host plant	Disease or antigen
Tobacco	38C13 mouse B-cell lymphoma
Tobacco	Anthrax
Tobacco	B-cell lymphoma
Tobacco	Breast and colon cancer
Tobacco	Broad-spectrum anticancer
Tobacco	Botulism
Tobacco	CD40 (cell surface protein)
Tobacco	Cell surface protein from mouse B-cell lymphoma
Tobacco	Hepatitis
Soybean	Herpes simplex virus
Tobacco	Human carcinoembryonic antigen
Pea	Human cancer cell surface antigen
Tobacco	Human CD40 cell surface protein
Tobacco	Human creatine kinase
Alfalfa	Human IgG
Tobacco	Rabies
Tobacco	*Salmonella* surface antigen
Tobacco	*Streptococcus mutans* cell surface antigen SA I/II
Tobacco	Substance P (neuropeptide)

Table 10.3

Table 10.3 Some potential vaccine antigens that have been expressed in plants

Disease or causative agent	Plant(s) or vector
Hepatitis B	Tobacco, potato, yellow lupin, lettuce
Malaria	Virus
Rabies	Tomato, spinach, virus
Human rhinovirus	Virus
HIV	Virus
E. coli	Tobacco, potato, corn
Norwalk virus	Tobacco, potato, corn
Diabetes	Tobacco, potato, carrot
Foot-and-mouth disease	*Arabidopsis*, alfalfa
Cholera	Potato, rice
Human cytomegalovirus	Tobacco
Dental caries	Tobacco
Respiratory syncytial virus	Tomato
Human papillomavirus	Potato, tobacco
Anthrax	Tobacco
SARS	Tomato, tobacco
Staphylococcus aureus	Cowpea
Measles	Lettuce
Influenza virus	Tobacco
Tuberculosis	*Arabidopsis*
Rotavirus	Alfalfa

Note that in some cases the antigen was cloned into a transient-expression system, such as a plant virus, often facilitating high levels of expression within a period of 1 to 2 weeks. SARS, severe acute respiratory syndrome.

Chapter 10
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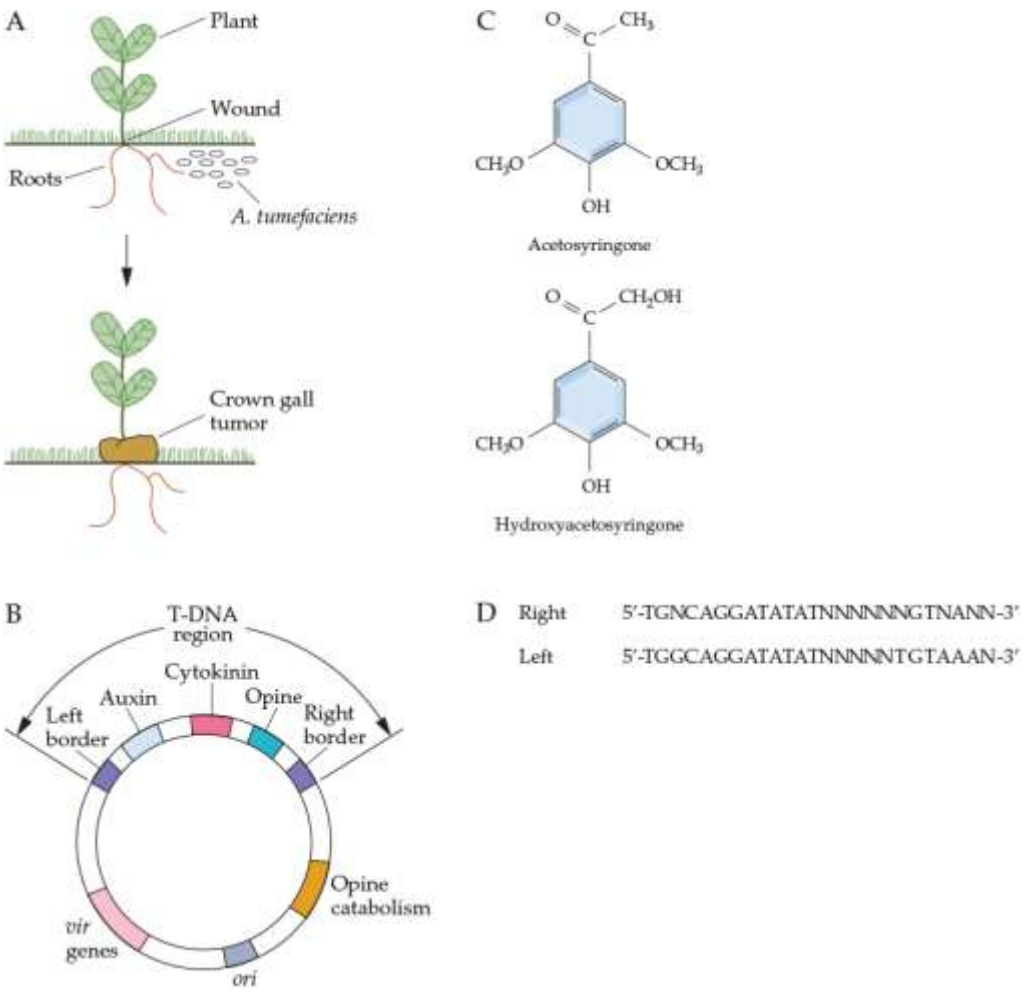
Figure 10.1 Genetic engineering of plants with the Ti plasmid of *Agrobacterium tumefaciens*, a soil bacteria.
A. Infection of a wounded plant by *Agrobacterium tumefaciens* leads to the production of a crown gall tumor (cancer)
B. The Ti plasmid of *Agrobacterium tumefaciens*
C. Wound signals produced by the plant to induce expression of the *vir* genes on the Ti plasmid
D. T-DNA right and left border nucleotide sequences involved in transfer of the T-DNA into the plant genome
See also https://www.youtube.com/watch?v=wTO-KmpZQgQ

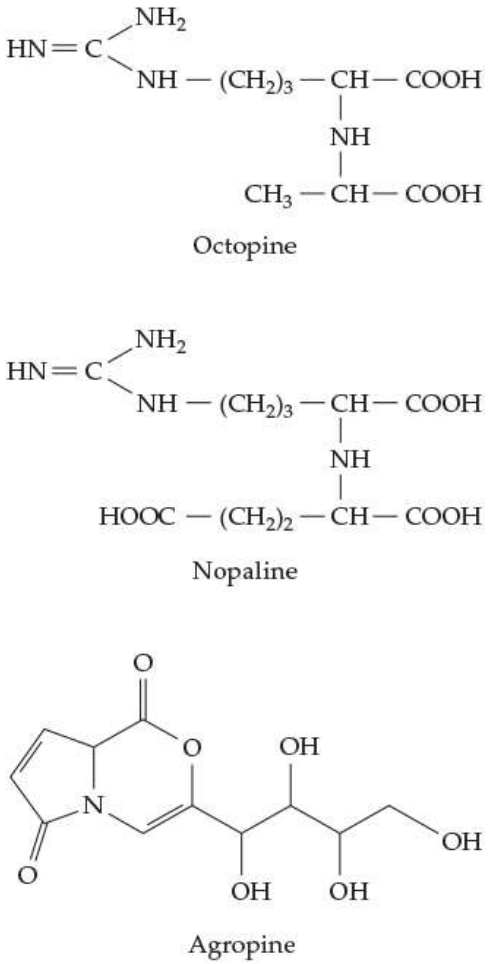


Molecular Biotechnology: Principles and Applications of Recombinant DNA, Fifth Edition
Bernard R. Glick, Cheryl L. Patten

Crown Gall on Tobacco

Infection of a plant with *Agrobacterium tumefaciens* and formation of crown galls

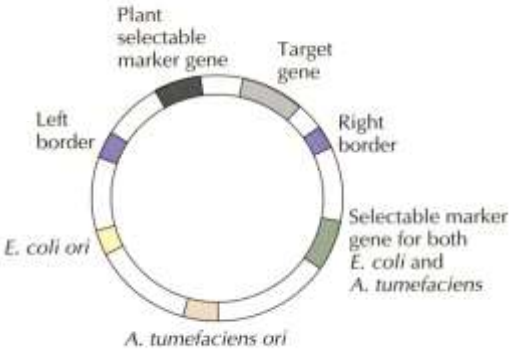
Figure 10.3 Chemical structures of three opines (amino acid-like molecules) produced by T-DNA genes expressed in an infected plant; opines are used as food by *A. tumefaciens*.



Plant Genetic Engineering with the binary Ti plasmid System

Clone YFG (your favorite gene) or a Target gene in the **small T-DNA-like plasmid** in *E. coli*, isolate this plasmid, and use it to transform *A. tumefaciens* which already contains a **disarmed Ti plasmid**. The disarmed Ti plasmid lacks a functional T-DNA region. This is a binary system as it involves 2 plasmids. Note that the *vir* genes on the disarmed Ti plasmid serve to move the T-DNA-like region of the small plasmid into the plant genome, resulting in random integration into the plant genome.

See also https://www.youtube.com/watch?v=L7qnY_GqytM



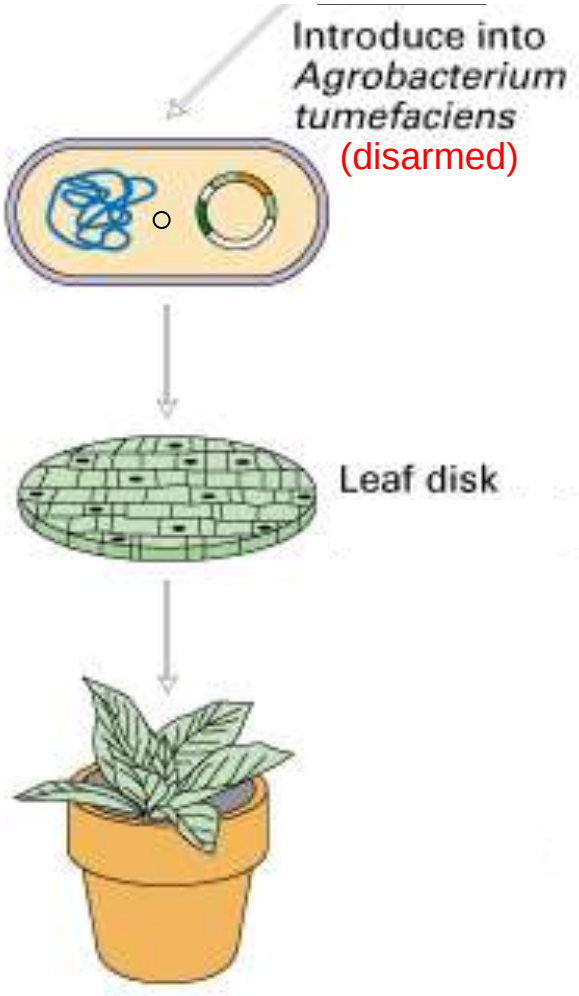


Figure 10.6 Using Microprojectile Bombardment (Biolistics) to shoot genes into plants, either into the plant nuclear genome or the plant chloroplast genome.

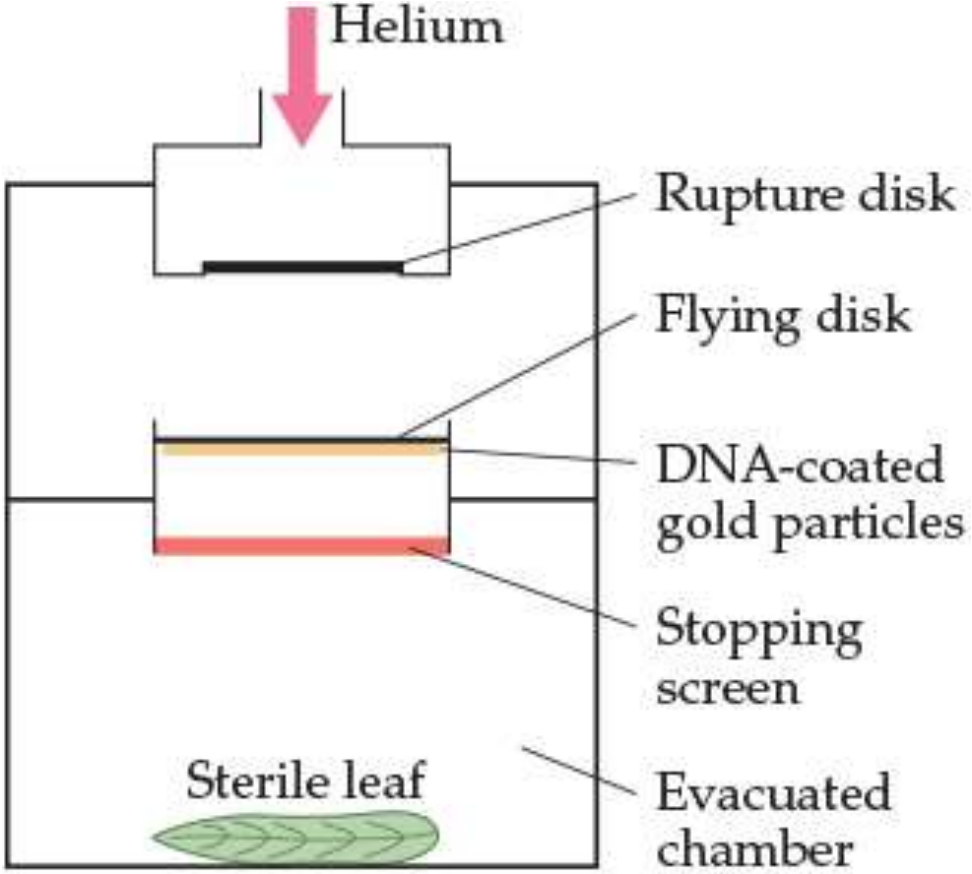


Figure 10.8 A. Gene constructions for:
A. Expression of a foreign (or target) gene in the nucleus and targeting of the foreign protein to the chloroplast and
B. Expression of a foreign (or target) gene in the chloroplast

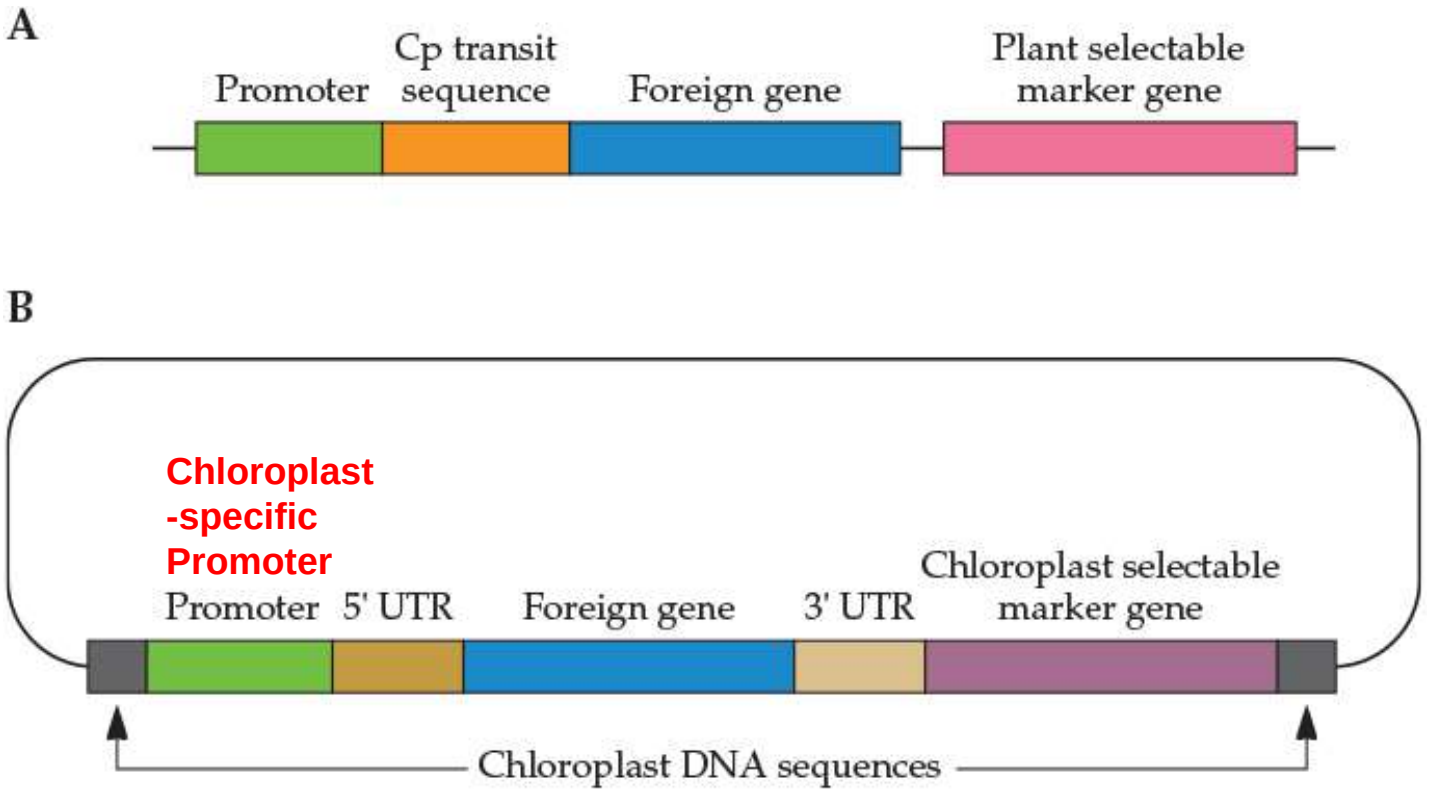


Table 10.5

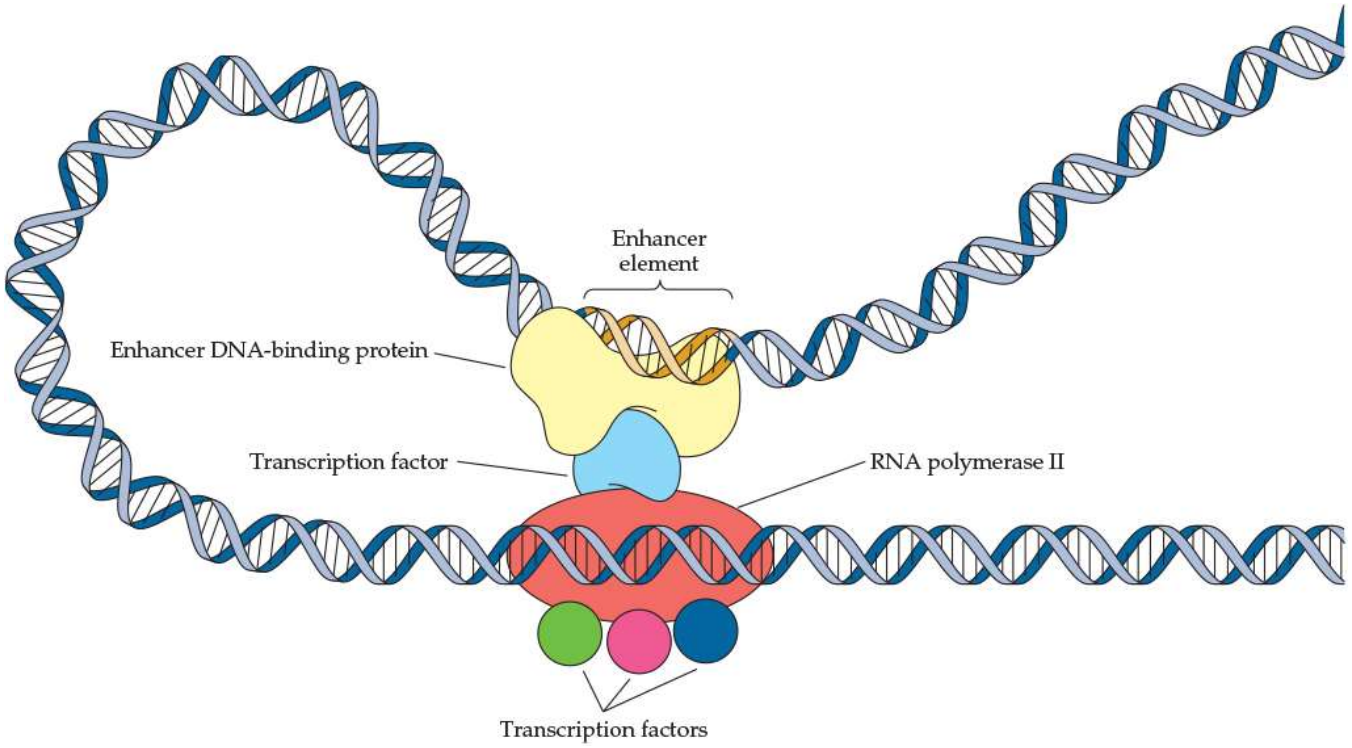
Table 10.5 Plant cell reporter and selectable marker gene systems

Enzyme activity	Selectable marker	Reporter gene
Neomycin phosphotransferase	Yes	Yes
Hygromycin phosphotransferase	Yes	Yes
Dihydrofolate reductase	Yes	Yes
Chloramphenicol acetyltransferase	Yes	Yes
Gentamicin acetyltransferase	Yes	Yes
Nopaline synthase	No	Yes
Octopine synthase	No	Yes
β-Glucuronidase	No	Yes
Streptomycin phosphotransferase	Yes	Yes
Bleomycin resistance	Yes	No
Firefly luciferase	No	Yes
Bacterial luciferase	No	Yes
Threonine dehydratase	Yes	Yes
Metallothionein II	Yes	Yes
enol-Pyruvylshikimate-3-phosphate synthase	Yes	No
Phosphinothricin acetyltransferase	Yes	Yes
β-Galactosidase	No	Yes
Blasticidin S deaminase	Yes	Yes
Acetolactate synthase	Yes	No
Bromoxynil nitrilase	Yes	No
Green fluorescent protein	No	Yes

Adapted from Walden and Schell, *Eur J Biochem* 192:563–576, 1990, and Gruber and Crosby, p. 89–119, in B. R. Glick and J. E. Thompson (ed.), *Methods in Plant Molecular Biology and Biotechnology* (CRC Press, Boca Raton, FL, 1993).

http://www.youtube.com/watch?v=Sl2PRHGpYuU

Figure 10.14 When expressing a target gene in plants, one needs to consider which plant promoter/enhancer sequence to use as this will determine where, when, and how much mRNA (and protein) is produced.



Manipulation of gene expression in plants: Choosing the right promoter/enhancer sequences to express your target gene

- Strong, constitutive promoters (35S Cauliflower mosaic virus promoter or 35S CaMV or 35S)
- Organ and tissue specific promoter (e.g., the leaf-specific promoter for the small subunit of the photosynthetic enzyme ribulosebisphosphate carboxylase or rbc)
- Promoterless reporter gene constructs to find new organ- and tissue-specific promoters
- Inducible promoters (Dex or dexamethasone)
- Synthetic promoters

Other DNA modules or genes to consider adding when expressing your target gene in plants

- Protein affinity tag sequences to facilitate purifying your target protein from plants (e.g., c-myc, FLAG, 6xHis, olesins [oil body proteins])
- Protease recognition sequences to remove affinity tags
- Signal peptide sequences to facilitate secretion of your target protein outside the cell (e.g., Rhizosecretion or secretion of your target protein by plant roots by using a a signal peptide sequence along with a root-specific promoter and growing the transgenic plant hydroponically (your target protein will be secreted into the hydroponic growth media)
- Cellular targeting sequences (nucleus, cell wall, ER, Golgi, mitochondria, chloroplasts, etc.)
- Genes which modify or inhibit certain plant protein glycosylation reactions to produce therapeutic glycoproteins with more human-like glycosylation

Figure 10.16 Fusing your target protein to oleosin (an oil body protein) to facilitate target protein purification.

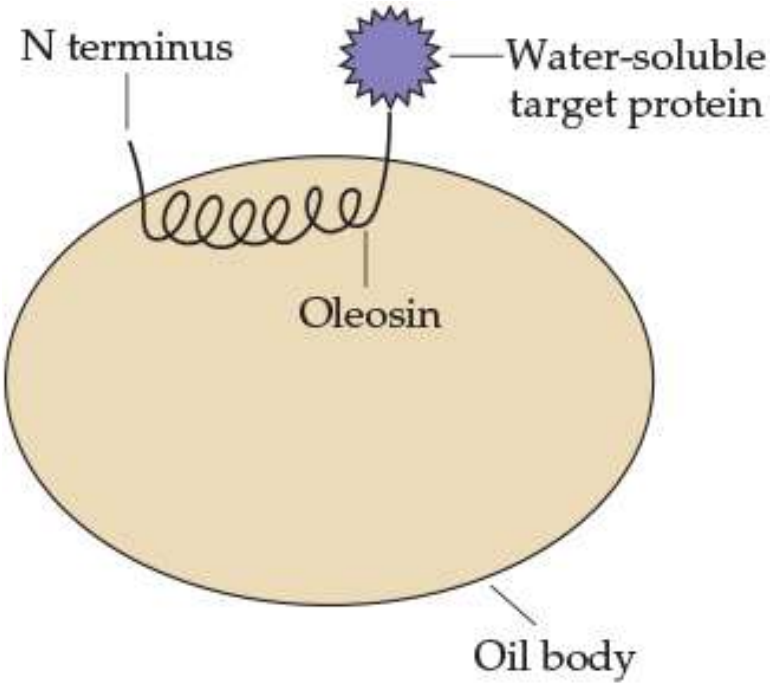


Figure 10.17 Expressing your target protein for secretion by the roots in a hydroponic system by a process called "rhizosecretion"

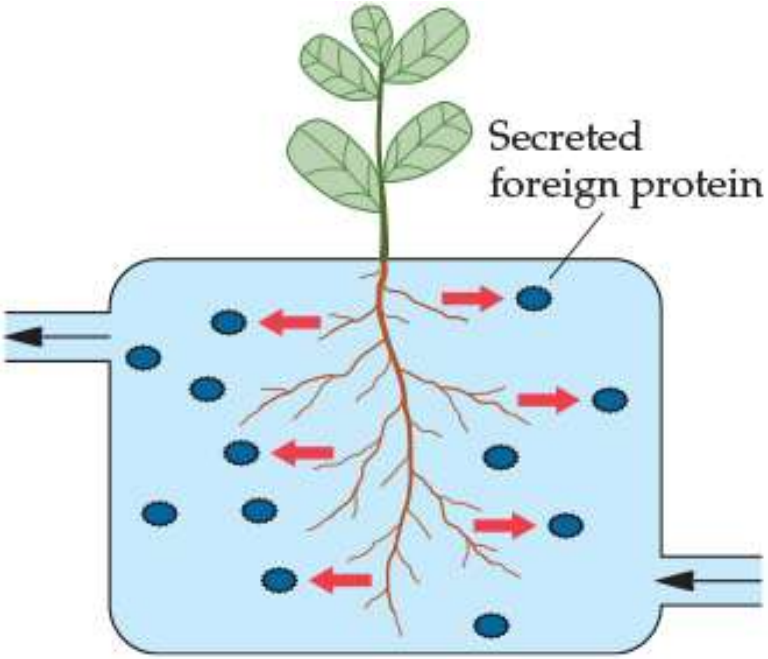


Figure 10.21 Removal of selectable marker sequences to produce "Marker-Free Transgenic Plants" to address potential human, animal, or environmental safety concerns. This can be done using A. A transposase gene and Ds elements surrounding the selectable marker or B. A recombinase gene and recombinase recognition sequences surrounding the selectable marker and the recombinase gene.