Biotechnology and Genetic Engineering

Recombinant DNA and DNA Science

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Biotechnology

- The use of living cells to make products such as pharmaceuticals, foods, and beverages
- The use of organisms such as bacteria to protect the environment
- The use of DNA science for the production of products, diagnostics, and research

Recombinant DNA

- The manipulation and combination of DNA from two sources
- Bacterial DNA + human gene for insulin
- Plant DNA + bacterial DNA - *Agrobacterium tumefaciens*
- Mouse DNA + human DNA = transgenic

Recombination

- Insert a foreign gene into a host Plasmid (for example, exogenous DNA) into the bacterial cell – transformation or transfection-organism referred to as transgenic (eukaryote) or recombinant(prokaryote)
- Goal – To produce many copies (clones) of a particular gene
- Reporter gene – tags gene of interest – to identify the presence of a gene

Vectors

- Plasmids
- Viruses
- Particles (DNA coated bullets)
- Exogenous DNA

Characteristics of a Vector

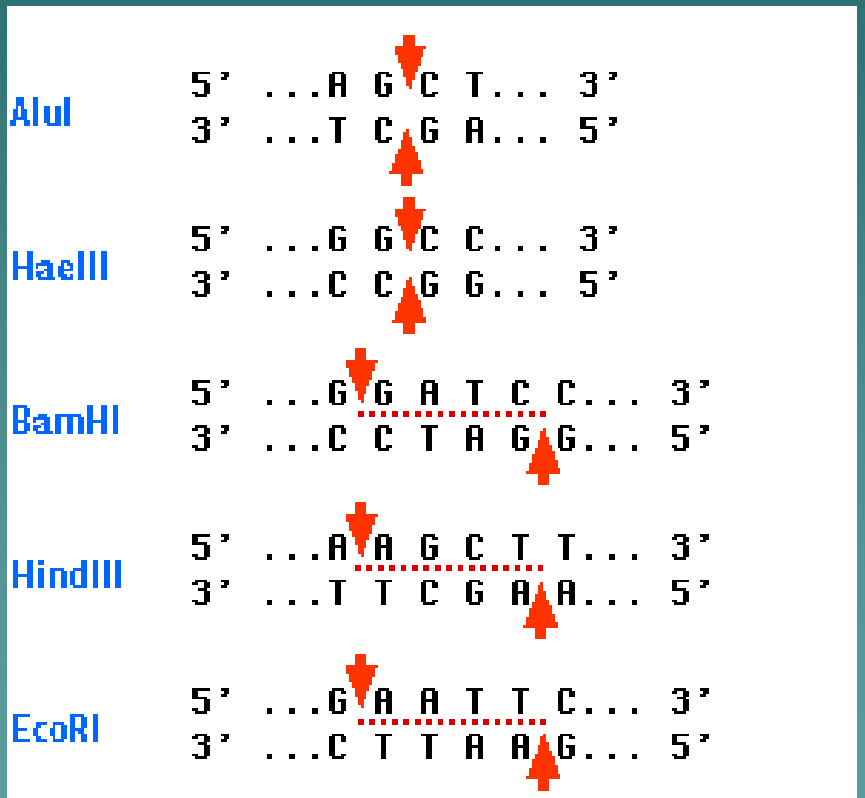
- Can replicate independently in the host cell – contains an Ori
- Has restriction sites in the vector- Polylinker cloning region
- Has a reporter gene that will announce its presence in the host cell
- Is a small size in comparison to the host chromosome for ease of isolation

Restriction Enzymes and Vectors

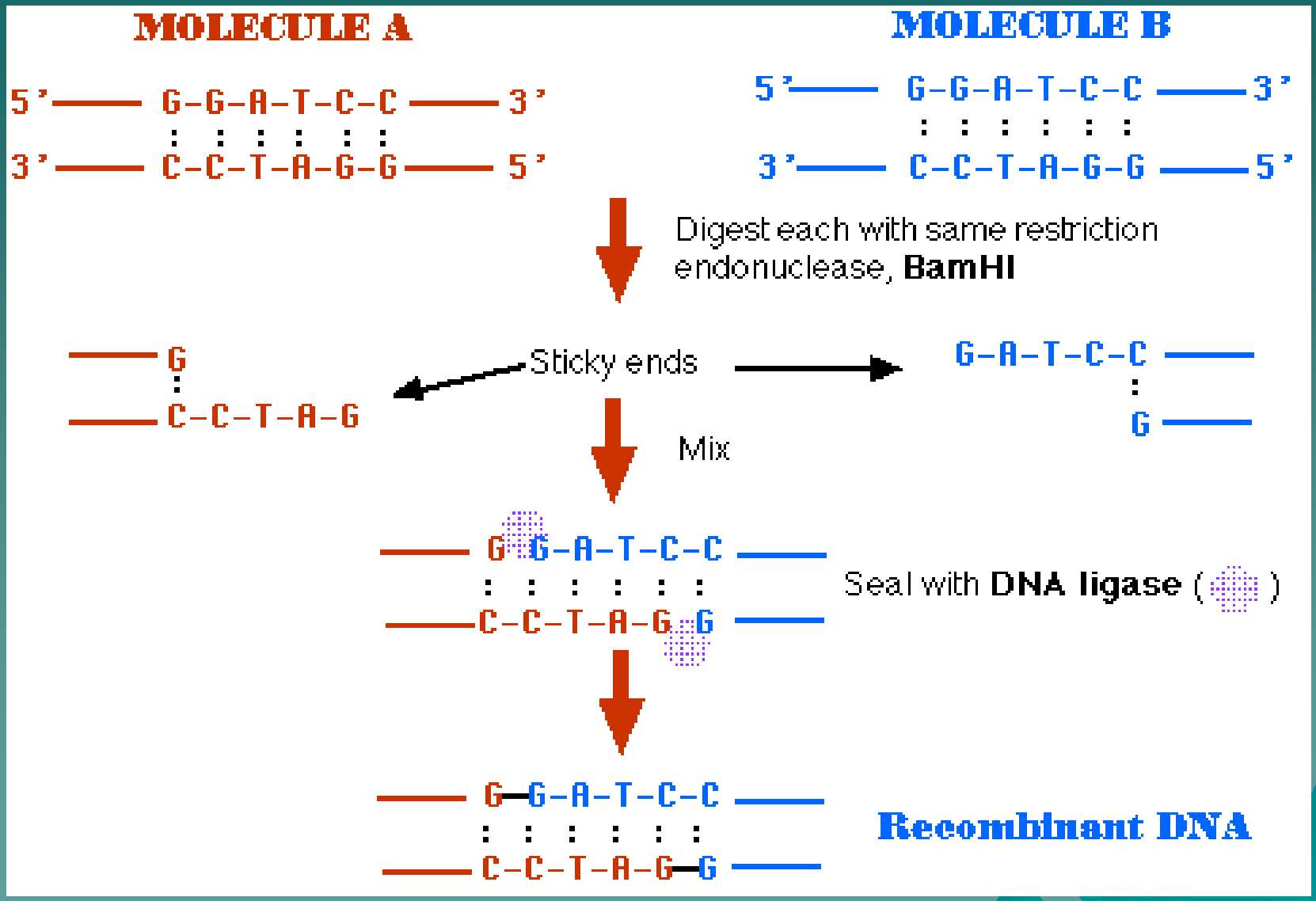
- Cut Plasmid with restriction enzyme
- Cut gene of interest with restriction enzyme
- Splice together gene of interest and vector

Tools for Recombination

- Restriction enzymes



Recombinant DNA

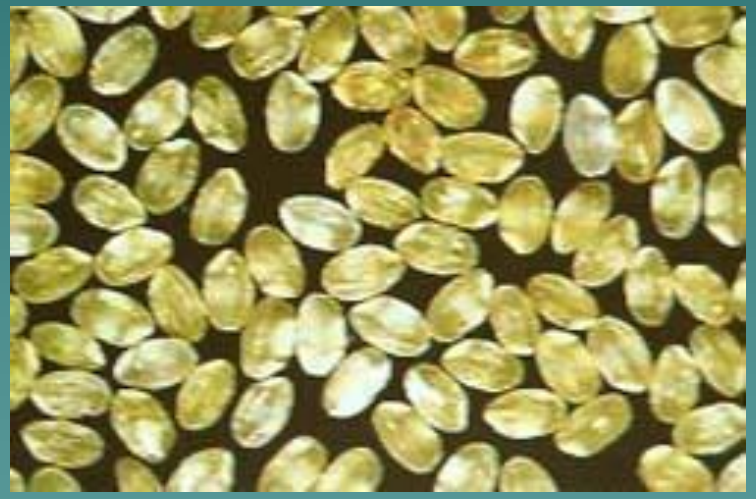


Pharmaceuticals

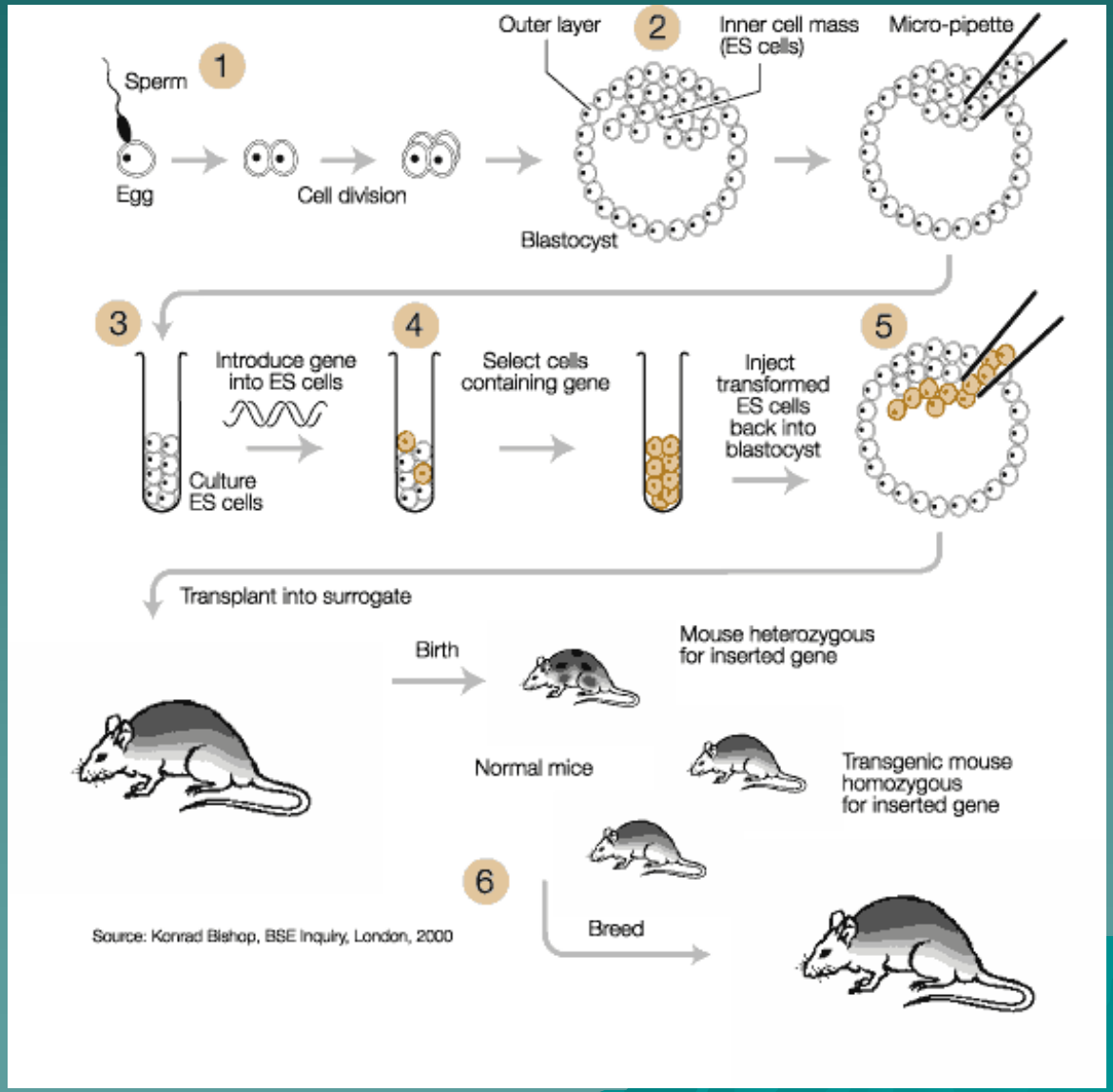
- **insulin** for diabetics
- **factor VIII** for males suffering from hemophilia A
- **factor IX** for hemophilia B
- **human growth hormone** (**GH**)
- **erythropoietin** (**EPO**) for treating anemia
- three types of **interferons**
- several **interleukins**
- **granulocyte-macrophage colony-stimulating factor** (**GM-CSF**) for stimulating the bone marrow after a bone marrow transplant
- **tissue plasminogen activator** (**TPA**) for dissolving blood clots
- **adenosine deaminase** (**ADA**) for treating some forms of **severe combined immunodeficiency** (**SCID**)
- **angiostatin** and **endostatin** for trials as anti-cancer drugs
- parathyroid hormone
- leptin
- hepatitis B surface antigen (**HBsAg**) to vaccinate against the hepatitis B virus

Golden Rice- Agrobiotech

- Golden rice is the result of an effort to develop rice varieties that produce provitamin-A (beta-carotene) as a means of alleviating vitamin A (retinol) deficiencies in the diets of poor and disadvantaged people in developing countries. Because traditional rice varieties do not produce provitamin-A, transgenic technologies were required.

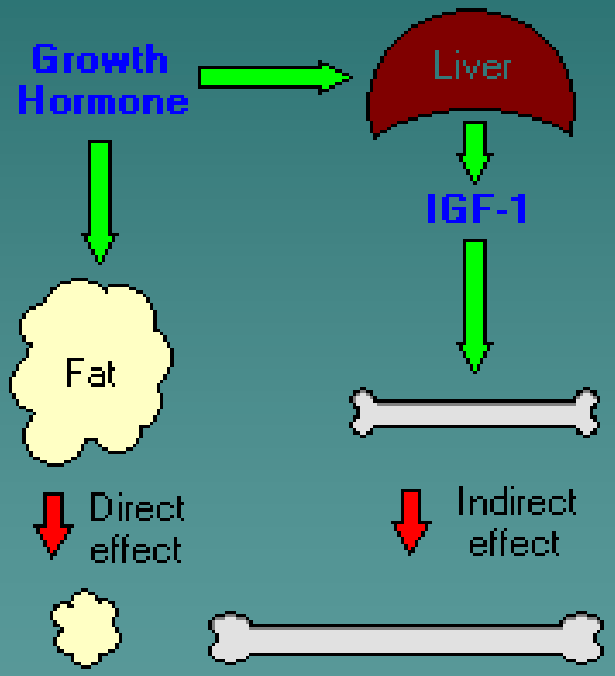


Transgenic Mice – Manipulation of embryo(blastocyst)



Growth hormone and therapy

- **Growth hormone, also known as *somatotropin*, is a protein hormone of about 190 amino acids that is synthesized and secreted by cells called *somatotrophs* in the anterior pituitary.** It is a major participant in control of several complex physiologic processes, including growth and metabolism. Growth hormone is also of considerable interest as a drug used in both humans and animals.

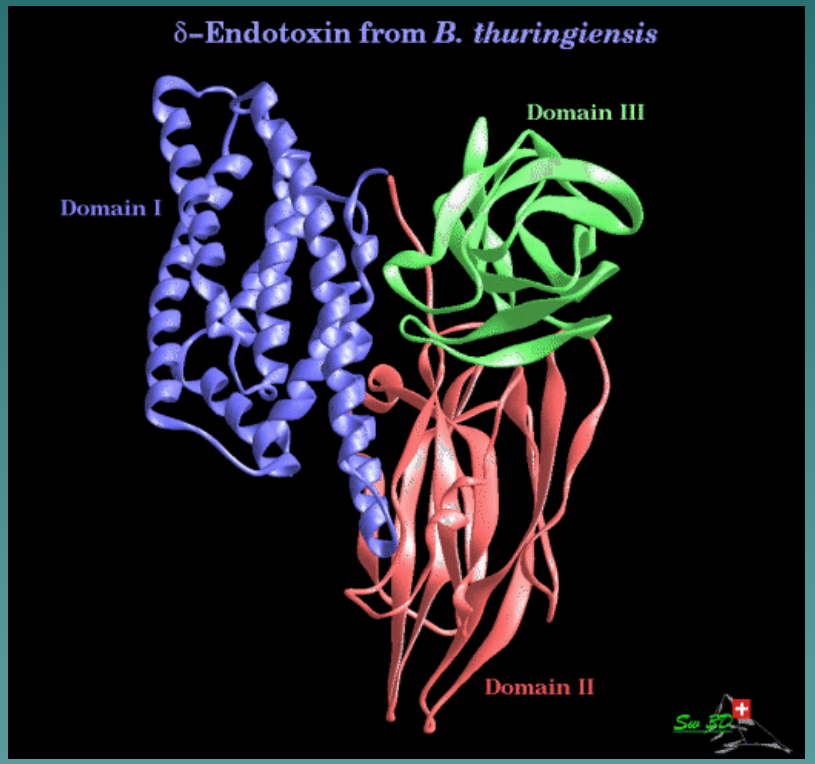


Transgenic mice

- Two baby mice - same age
- Human Growth hormone inserted into the embryo of the mouse on the left. Causes rapid growth in the newborn
- The mouse on the right is a normal sized mouse

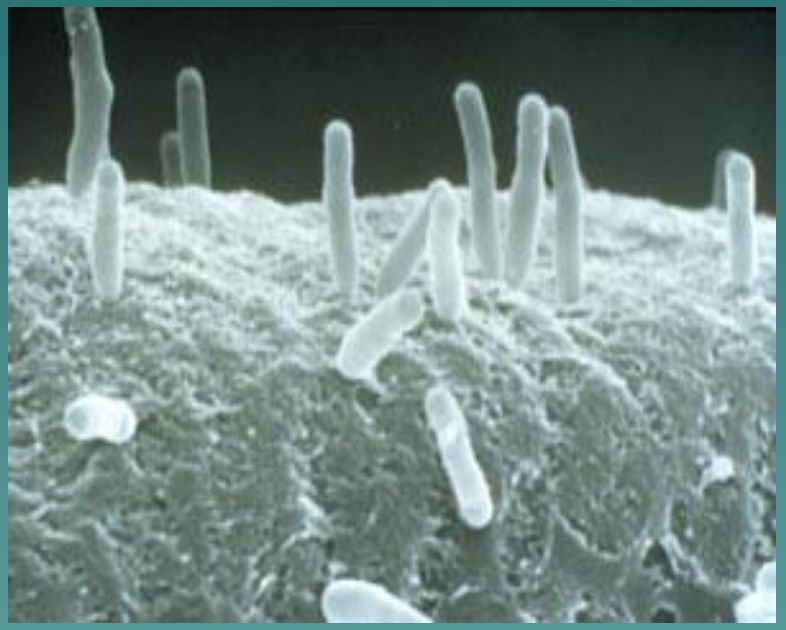
Insect Resistance

- *B. thuringiensis* (commonly known as 'Bt') is an insecticidal bacterium, marketed worldwide for control of many important plant pests - mainly caterpillars of the Lepidoptera (butterflies and moths) but also mosquito larvae, and simuliid blackflies that vector river blindness in Africa. Bt products represent about 1% of the total 'agrochemical' market (fungicides, herbicides and insecticides)

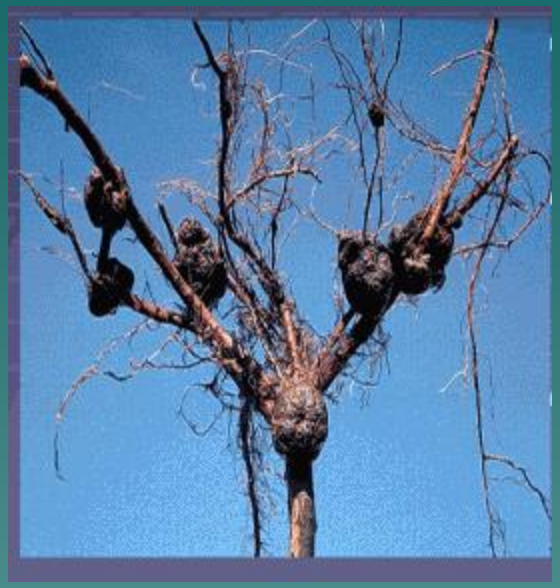


Agrobacterium tumefaciens

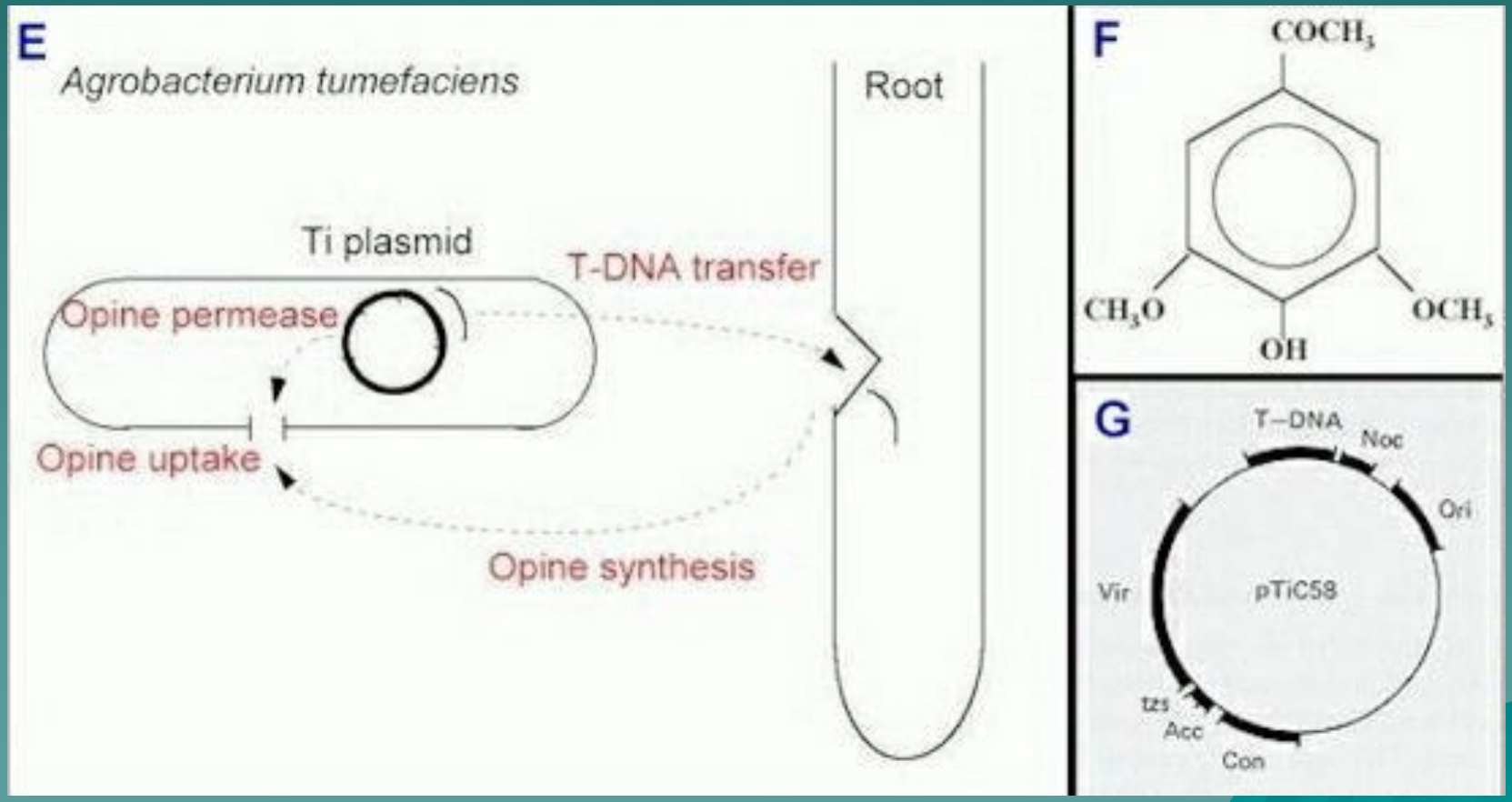
- *Agrobacterium tumefaciens* causes crown gall disease by first transferring part of its DNA into an opening in the plant. The DNA then integrates itself into the plant's genome and causes the formation of the gall.



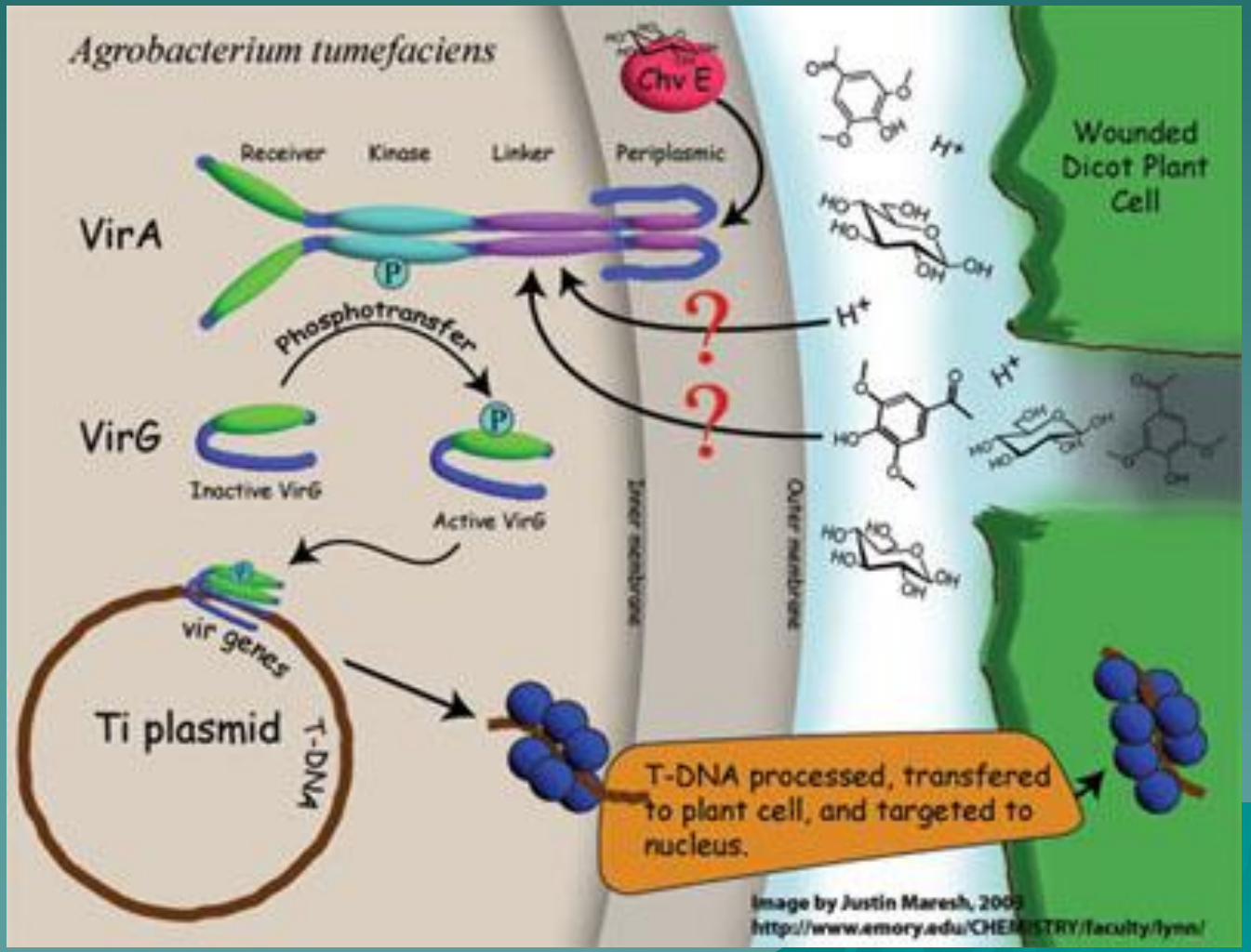
Crown Gall – Plant tumor



Nature's Genetic Engineering

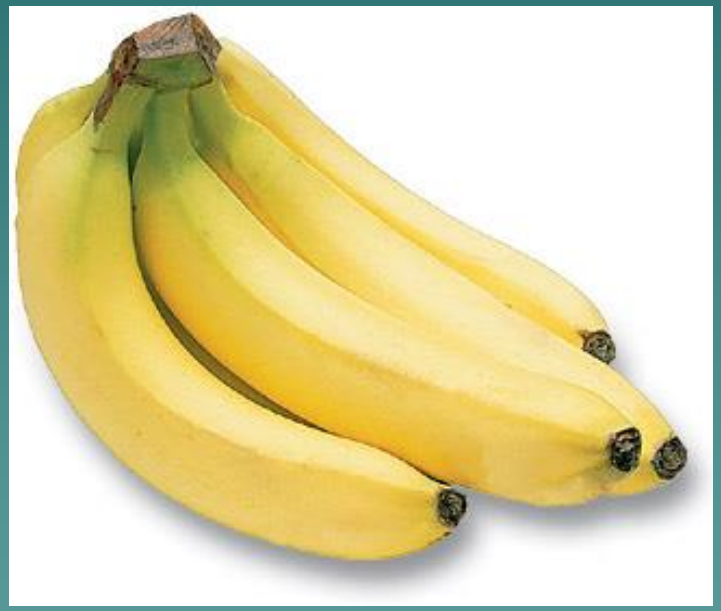


Agrobacterium tumefaciens

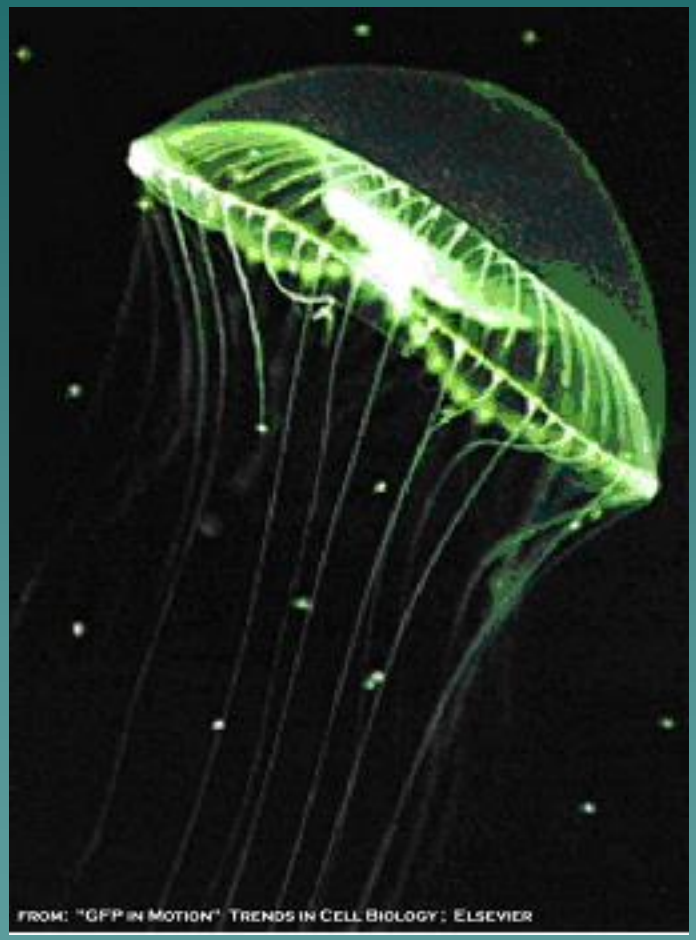


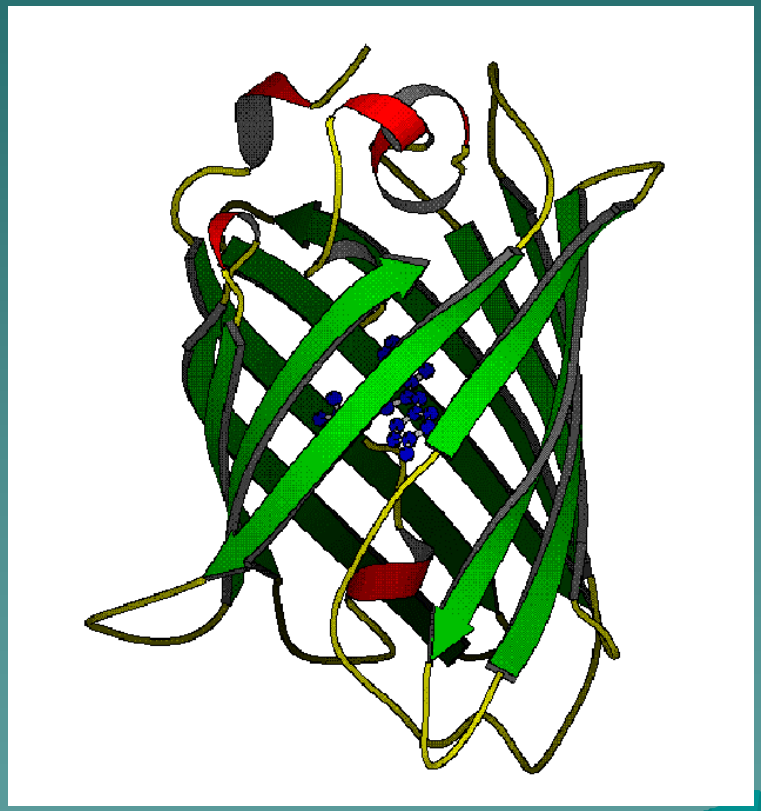
Vaccines

- Bananas have potential to become the world's first edible vaccine due to *Agrobacterium*. An edible vaccine doesn't need sterile syringes, costly refrigeration, or multiple injections. According to the World Health Organization (WHO), more than 2 million children die worldwide each year from diarrhea that can be prevented easily with vaccines. Thus, researchers lead by Dr. Charles Arntzen are looking into making the food vaccines to prevent diarrhea caused by *Escherichia coli* and *Vibrio cholara* bacteria.



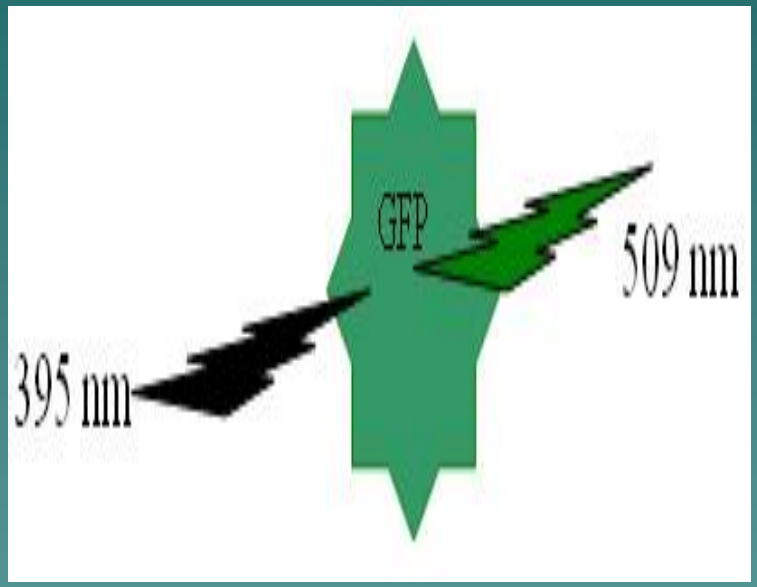
pGlo – Gfp
Green fluorescent protein





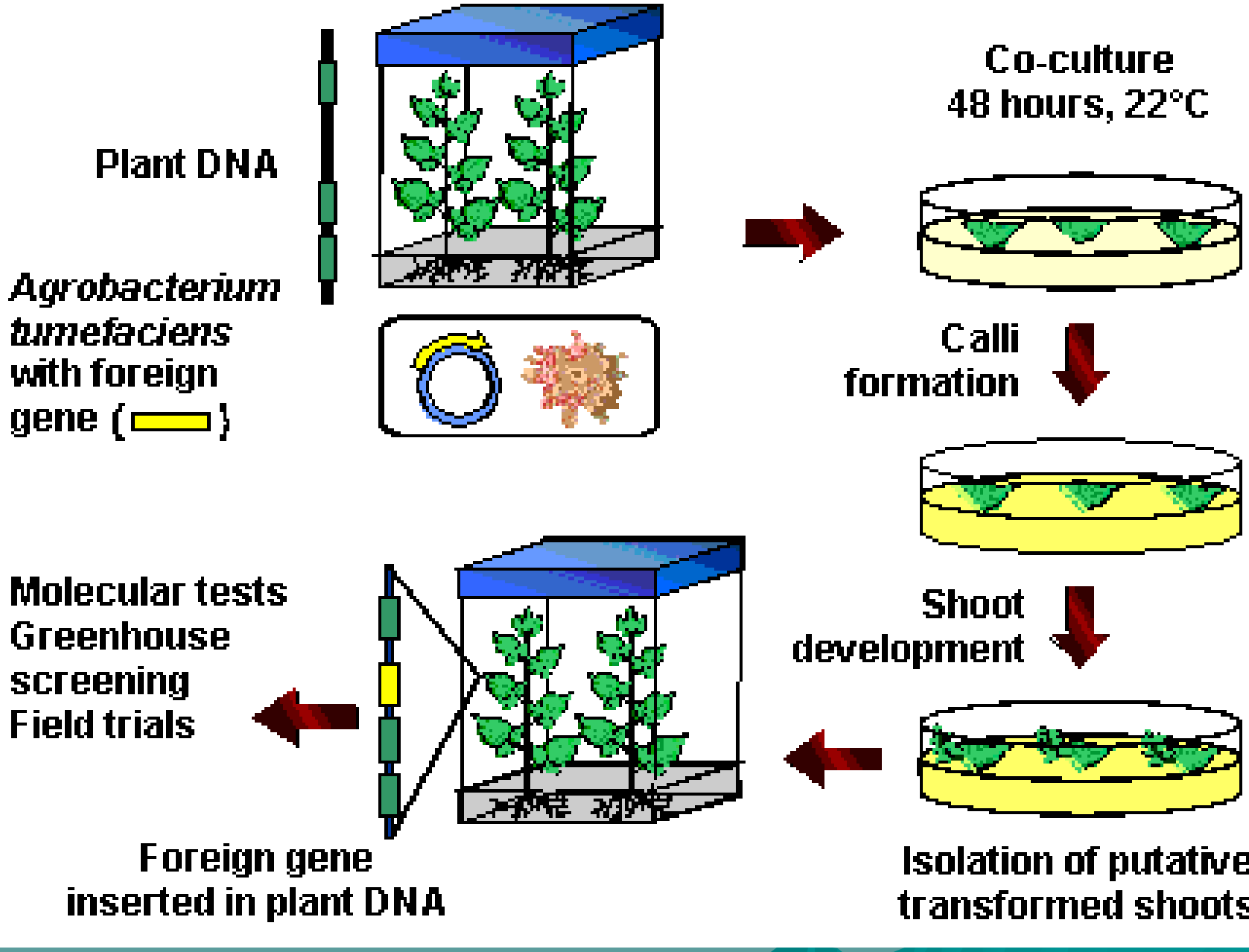
Fluorescent

- In the laboratory, fluorescence is easily achieved by exposing the protein to long range UV light or " black" light.
- The fluorophore absorbs light in the UV-B region (395 nm.. plus a smaller absorbance peak at 470 nm)
- It emits light (fluoresces) at 509 nm, which is in the green part of the visible spectrum

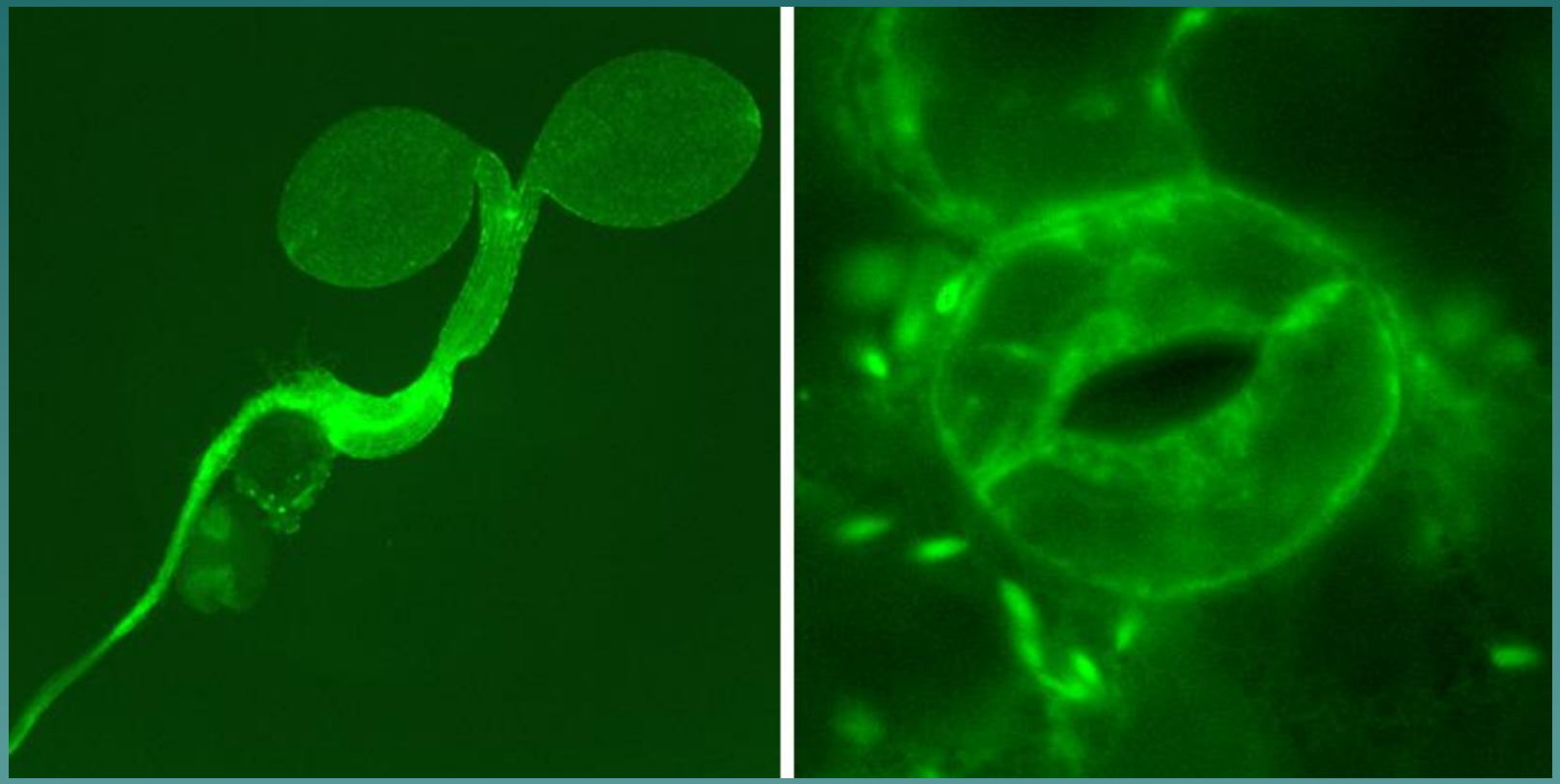


Gfp and Land Mines

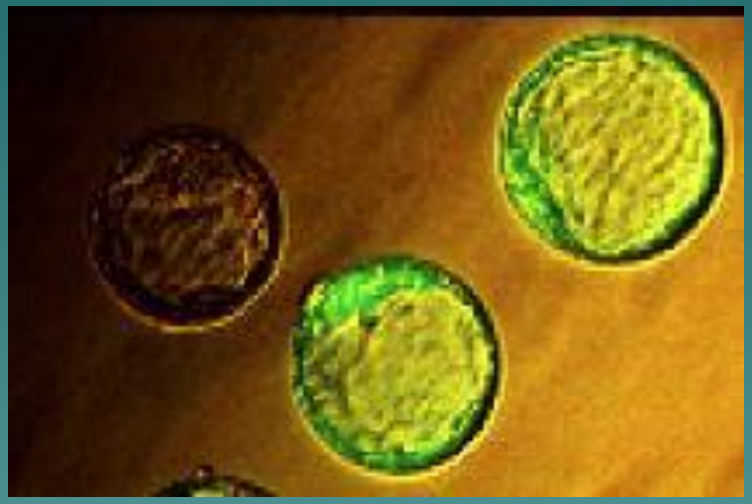
- Neal Stewart at the University of North Carolina is developing plants that can detect land mines
- Plants could be ideal biosensors for land mines as seeds would be spread widely and evenly in a suspect field
- The gene that can announce the presence of land mines is gfp
- The gene will be expressed in the presence of a land mine

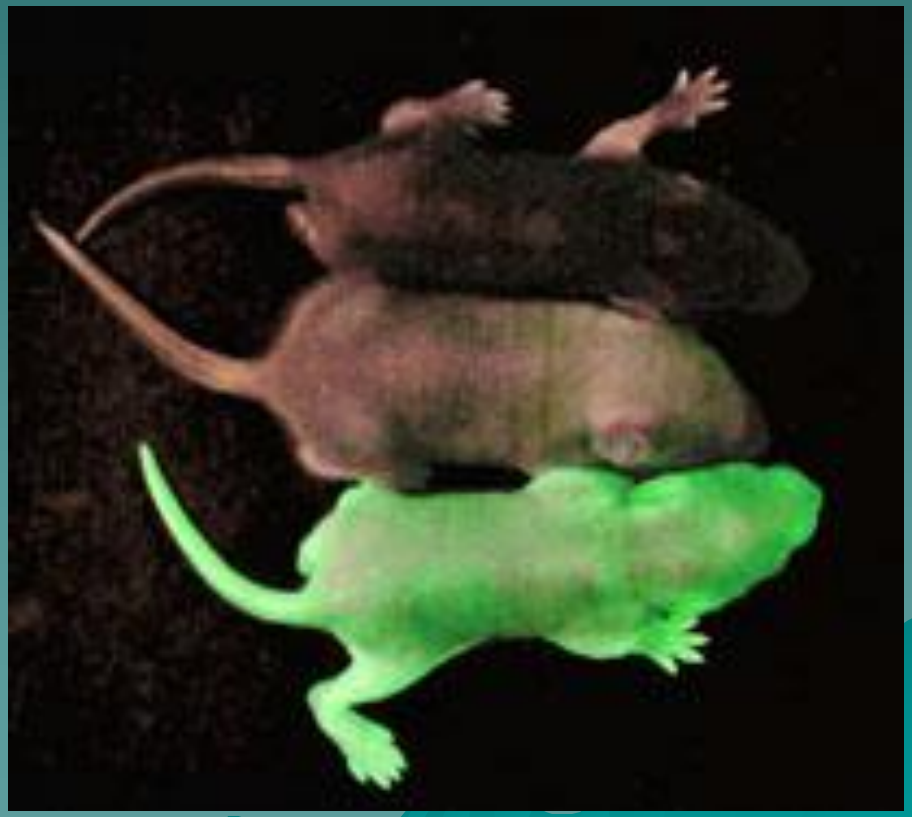

Plant DNA
Agrobacterium tumefaciens with foreign gene
Co-culture
48 hours, 22°C
Calli formation
Shoot development
Isolation of putative transformed shoots
Molecular tests
Greenhouse screening
Field trials
Foreign gene inserted in plant DNA

Green Fluorescent Protein and Plants



GFP and mice



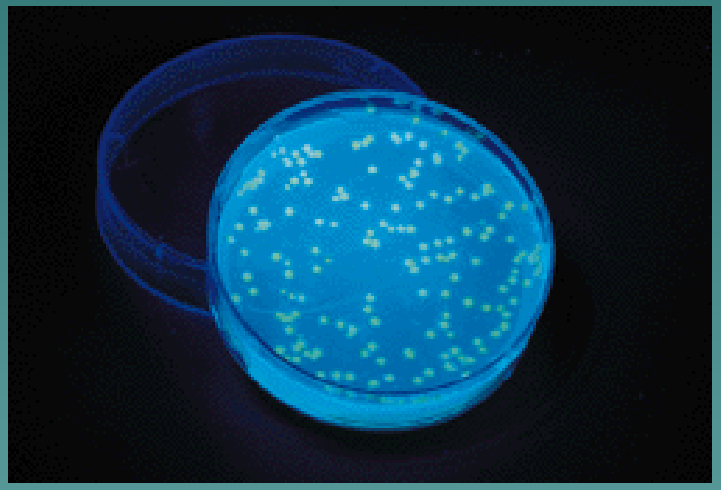


Glo fish

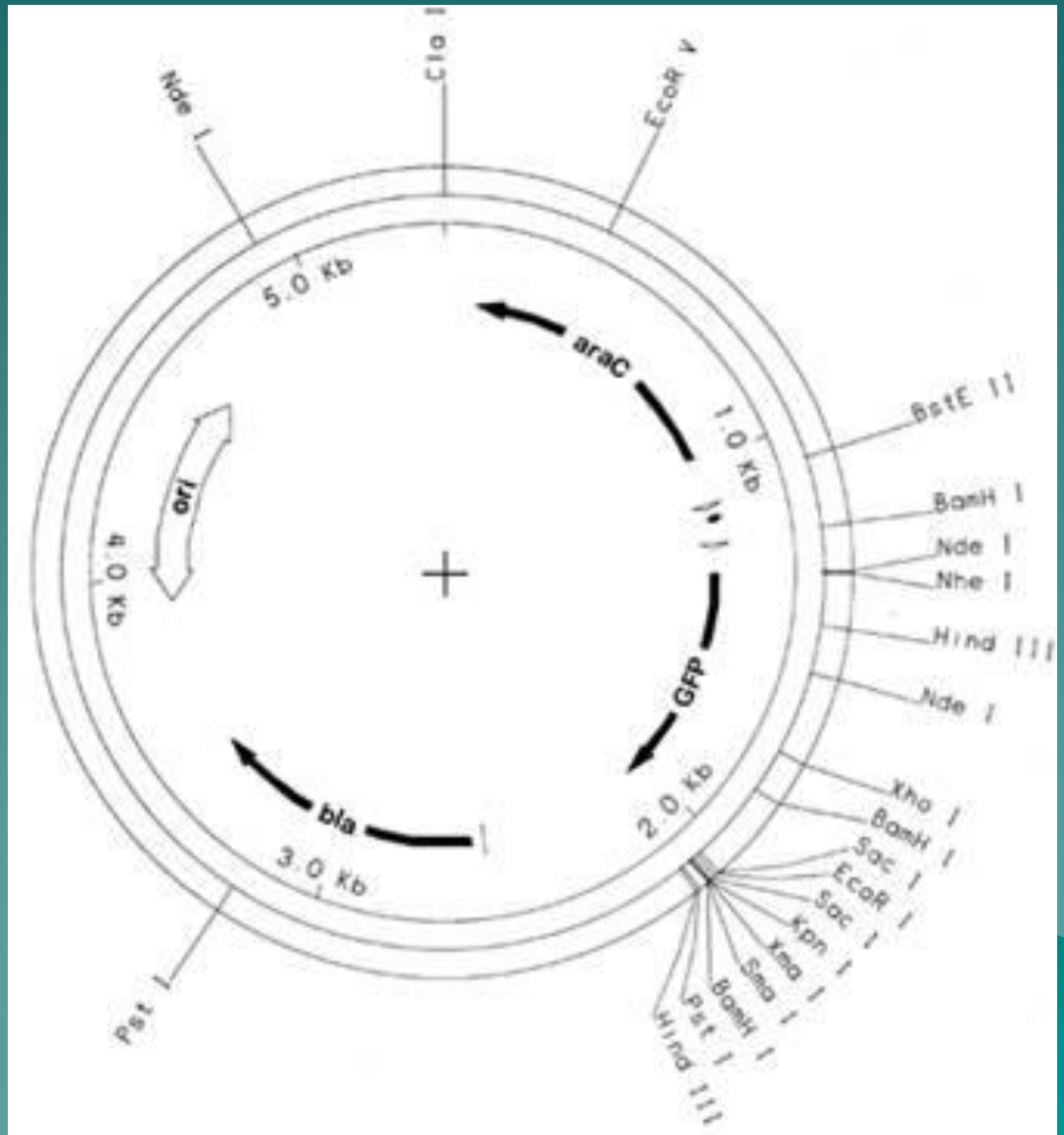
- Fluorescent zebra fish were specially bred to help detect environmental pollutants. By adding a natural fluorescence gene to the fish, scientists are able to quickly and easily determine when our waterways are contaminated

pGlo

- Transformation of *E. coli* with the pGlo plasmid
- Ori
- Gene for Gfp
- The plasmid contains the genes for the Arabinose promoter
- The plasmid contains the genes for ampicillin resistance
- If the bacterium uptakes the plasmid it should glow in response to long range uv light



pGlo


Nde I
Cla I
EcoR V
5.0 Kb
araC
1.0 Kb
BstE II
BamH I
Nde I
Nhe I
Hind III
Nde I
ori
4.0 Kb
GFP
2.0 Kb
Xho I
BamH I
Sac I
EcoR I
Sac I
Kpn I
Xma I
Sma I
BamH I
Pst I
Hind III
bla
3.0 Kb
Pst I

Arabinose operon

- ***araO1*** is an operator site. ***AraC*** binds to this site and represses its own transcription from the **PC** promoter. In the presence of arabinose, however, ***AraC*** bound at this site helps to activate expression of the **PBAD** promoter.

- ***araO2*** is also an operator site. ***AraC*** bound at this site can simultaneously bind to the ***araI*** site to repress transcription from the **PBAD** promoter

- ***araI*** is also the inducer site. ***AraC*** bound at this site can simultaneously bind to the ***araO2*** site to repress transcription from the **PBAD** promoter. In the presence of arabinose, however, ***AraC*** bound at this site helps to activate expression of the **PBAD** promoter.

Trasnformation movie

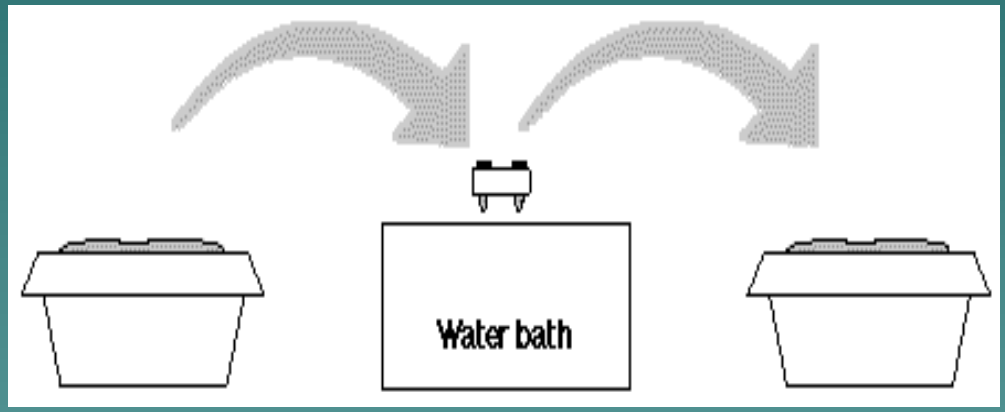
- Transformation movie

P Glo transformation

- Pick one colony from the starter plate.
- Use the sterile loops
- Swirl the loop in ice cold CaCl2 (experimental)
- Place in ice for 10 minutes (Your tubes will be incubating when you enter the room). I have found that a longer incubation period here increases the yield of transformants
- While the tubes are incubating label your plates
- LB AMP these plates eliminate bacteria that do not have gene for antibiotic resistance to ampicillin
- LB/AMP? Ara- These plates contain Arabinose and Ampicillin
- These are called the selection plates. The Arabinose will induce the gene to be turned on
- LB- Luria Broth Agar – all bacteria should grow on this agar

Heat Shock

- Leave cells in transformation solution on ice for ten minutes
- Transfer to water bath at 42oC for 90 seconds
- Return cells to ice



Lac Operon

- Operons –Organization of genes for metabolic pathways in bacteria
- Lac Operon - All genes for the metabolism of lactose connected with one Open reading frame or ORF
- Promoter for binding RNA polymerase for transcription of the gene
- Repressor molecule turns off transcription by binding to an operator next to the start 3' TAC

Recovery and Plating

- Incubate bacteria in Luria Broth for 10 minutes before plating in Petri Dish
- Plate your bacteria

+ pGlo – LB AMP and LB/Amp/Ara

- pGlo – LB and LB/AMP

Gene Expression and Genetic Engineering

- Links
- Operon movie in Quick Time
- Lac Operon
- Trp Operon Movie
- E. coli gene regulation
- Gene Regulation