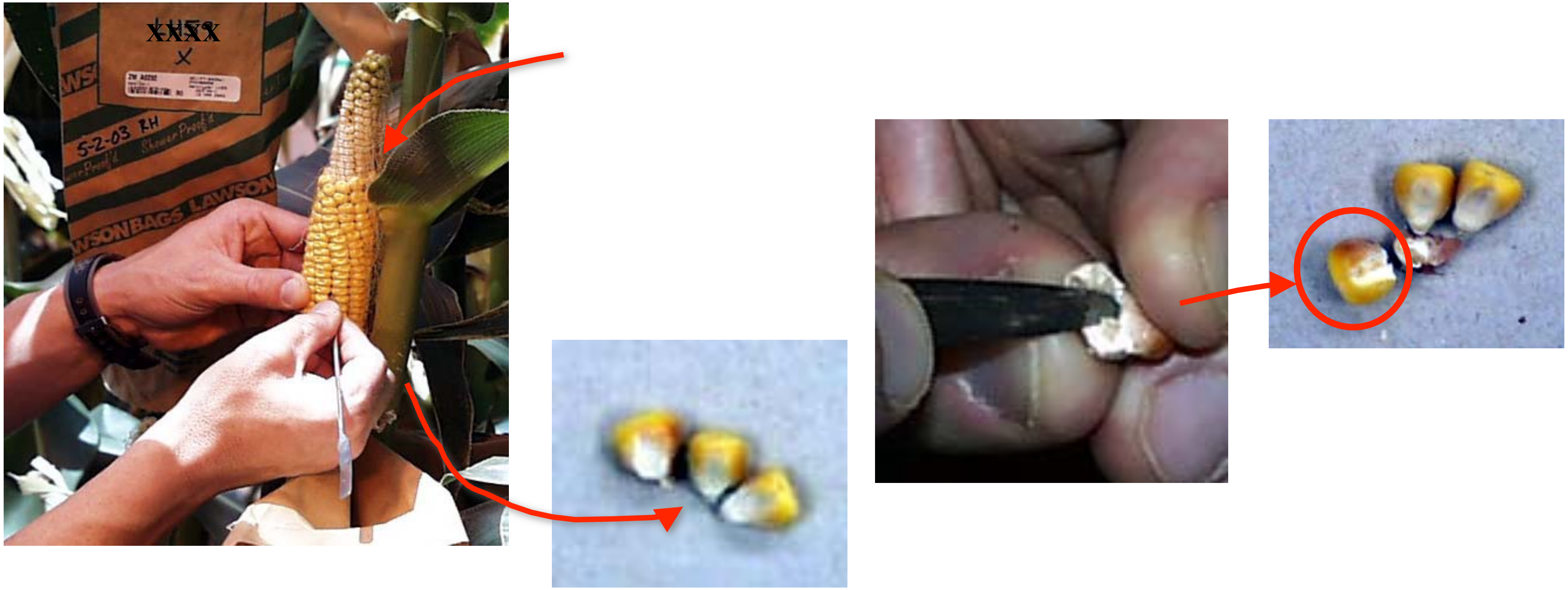
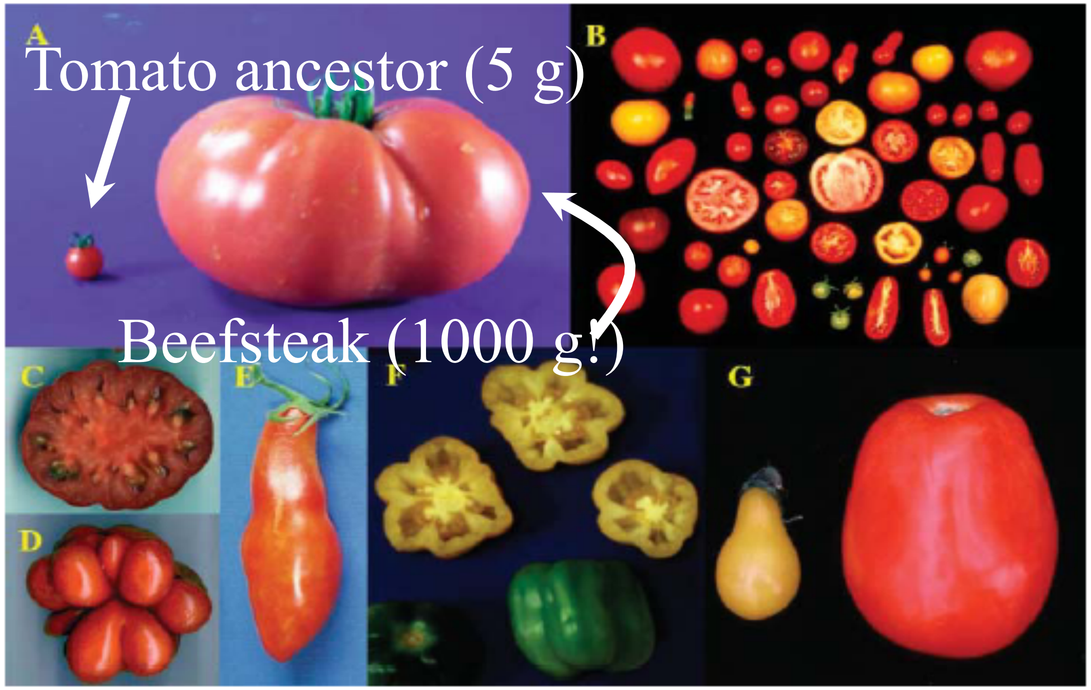


Plant biotechnology:
what it means and where we’re going

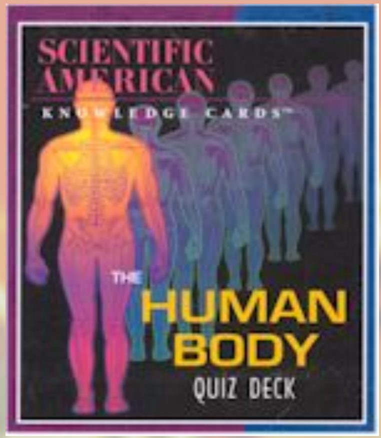
Biotechnology has a long history

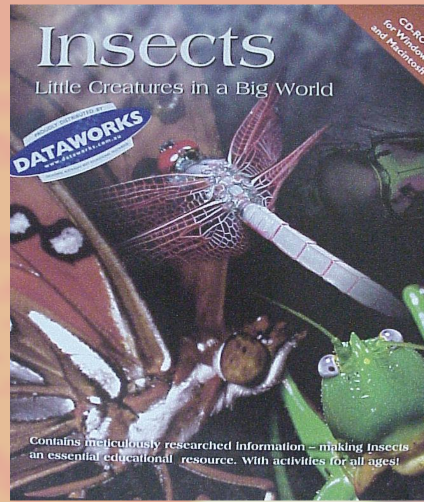
- Microbial/yeast biotech has long history: making cheese, bread, beer, wine
- "old-style" plant biotech has been used for crop improvement for centuries
  - Select mutants for best yield and quality (tomatoes!)
  - Breed plants to further improve desirable characteristics
  - Ultimate improved crop is maize: from from teosinte



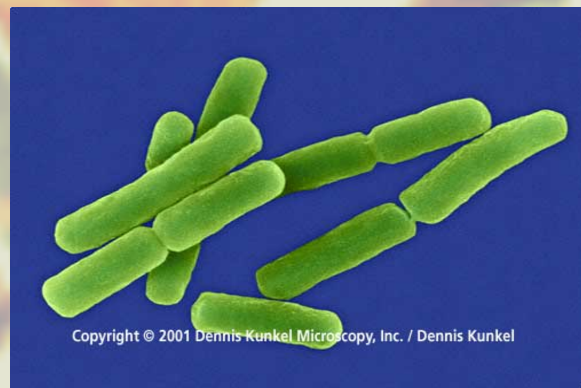
What is plant biotechnology?

- Manipulating plants and plant parts for practical uses
  - Improved food crops
    - Higher yields
    - Improved nutrition
    - Environmental tolerances
  - Improved production of valuable molecules
  - Production of novel molecules



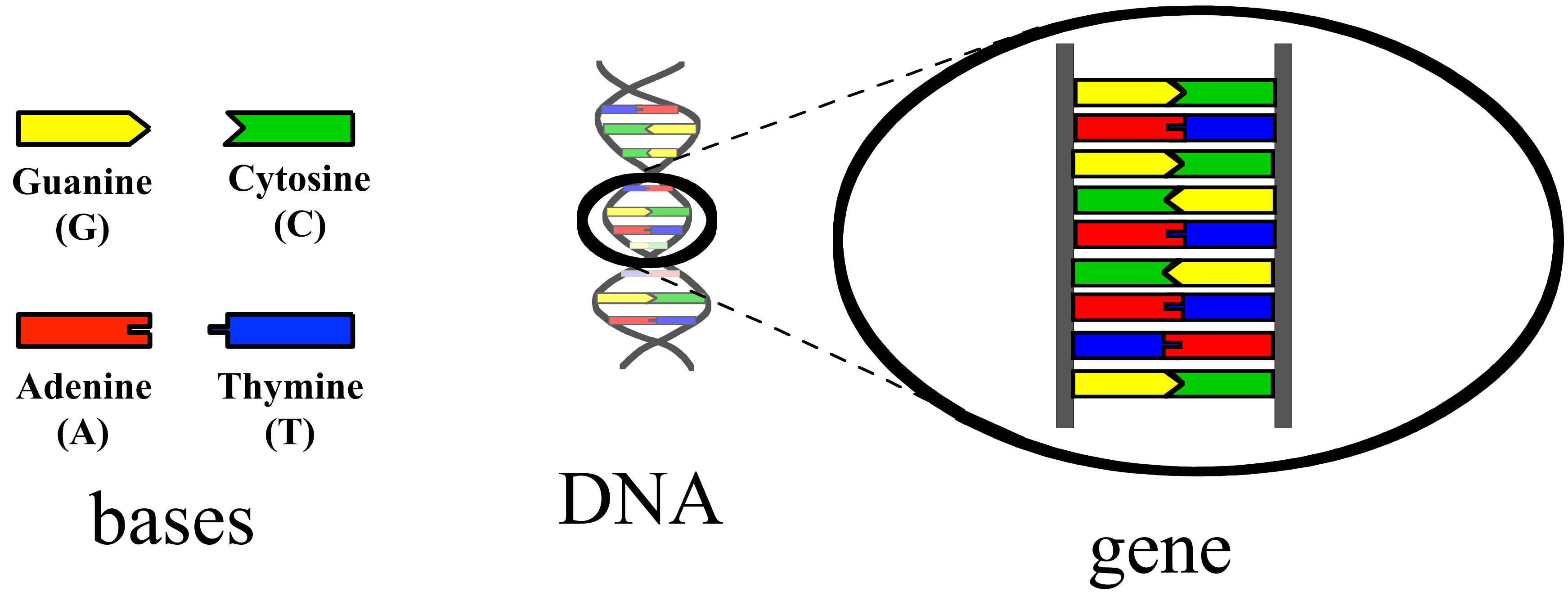


DNA IS EVERYWHERE



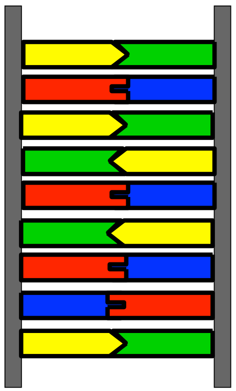
DNA and Genes

- ***Genes*** = the coding system for instructions
- ***A gene*** = is a segment of **DNA**



Genes and Proteins

transcription



Gene
(a piece of DNA)

→ mRNA → protein ⋯→ trait

translation

Biotechnology's four concepts

- All organisms are made of cells and cell products
- Each cell in an organism contains the same set of genes
- The genome contains all the genetic information necessary to make an entire organism
- All organisms share the same genetic language

Biotechnology in agriculture has two categories:

- **"Improvements" to existing livestock and crops, and**
- **Development of entirely new uses for both animals and plants (biopharming).**

"Improvements", include 'input traits' such as crops with extra resistance to insect attack, improved weed control, increase the plants tolerance to cold, drought and other environmental factor.

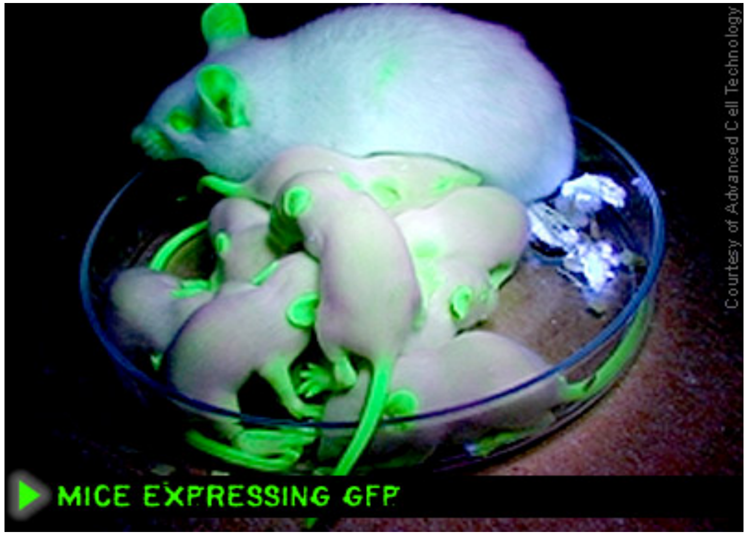
Biotechnology & agriculture

- Terminology
  - Transgenic
  - GMO
  - GM crop

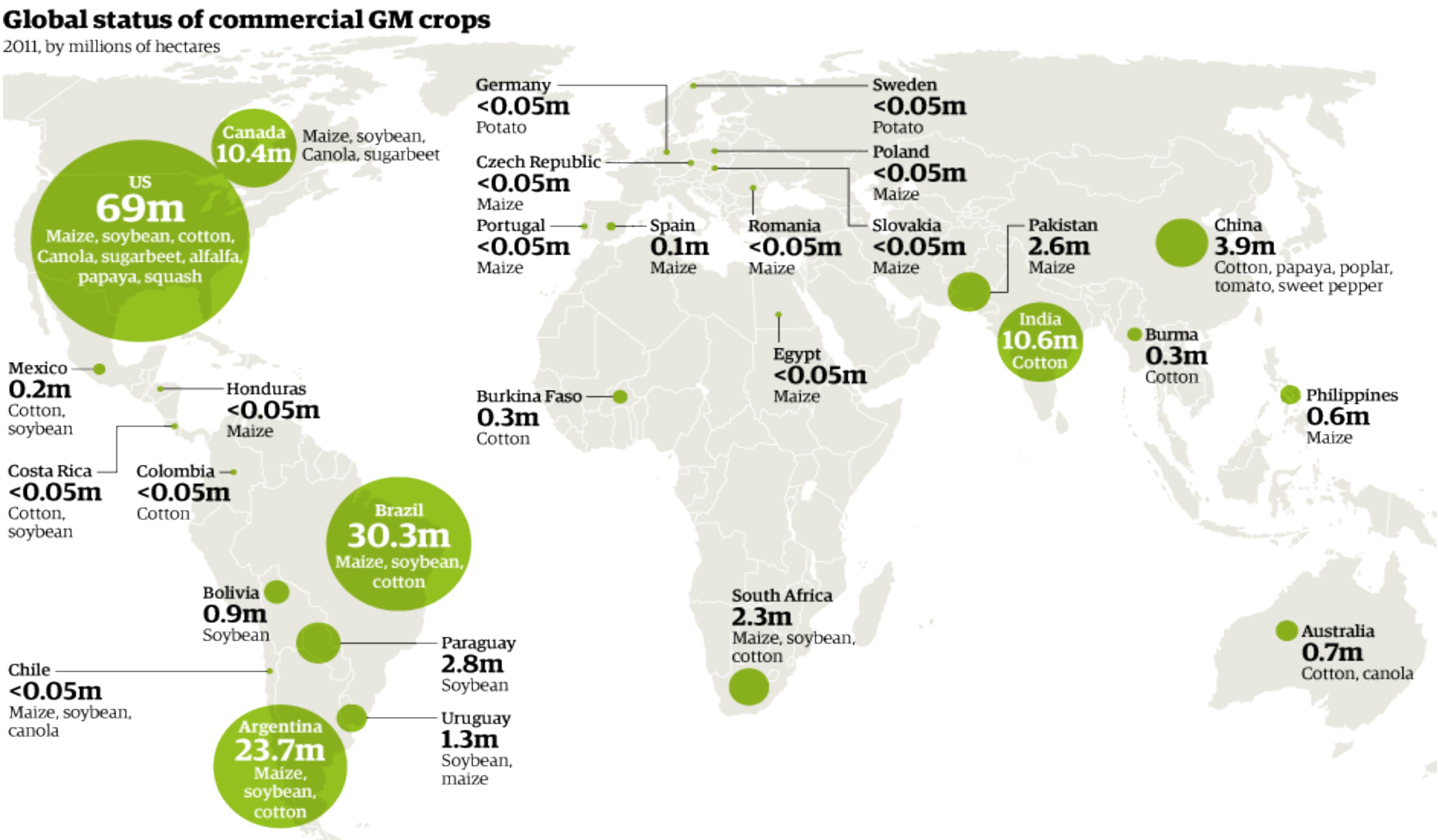
ng as Benjamin Cummings.

What is a Genetically Modified Organism?

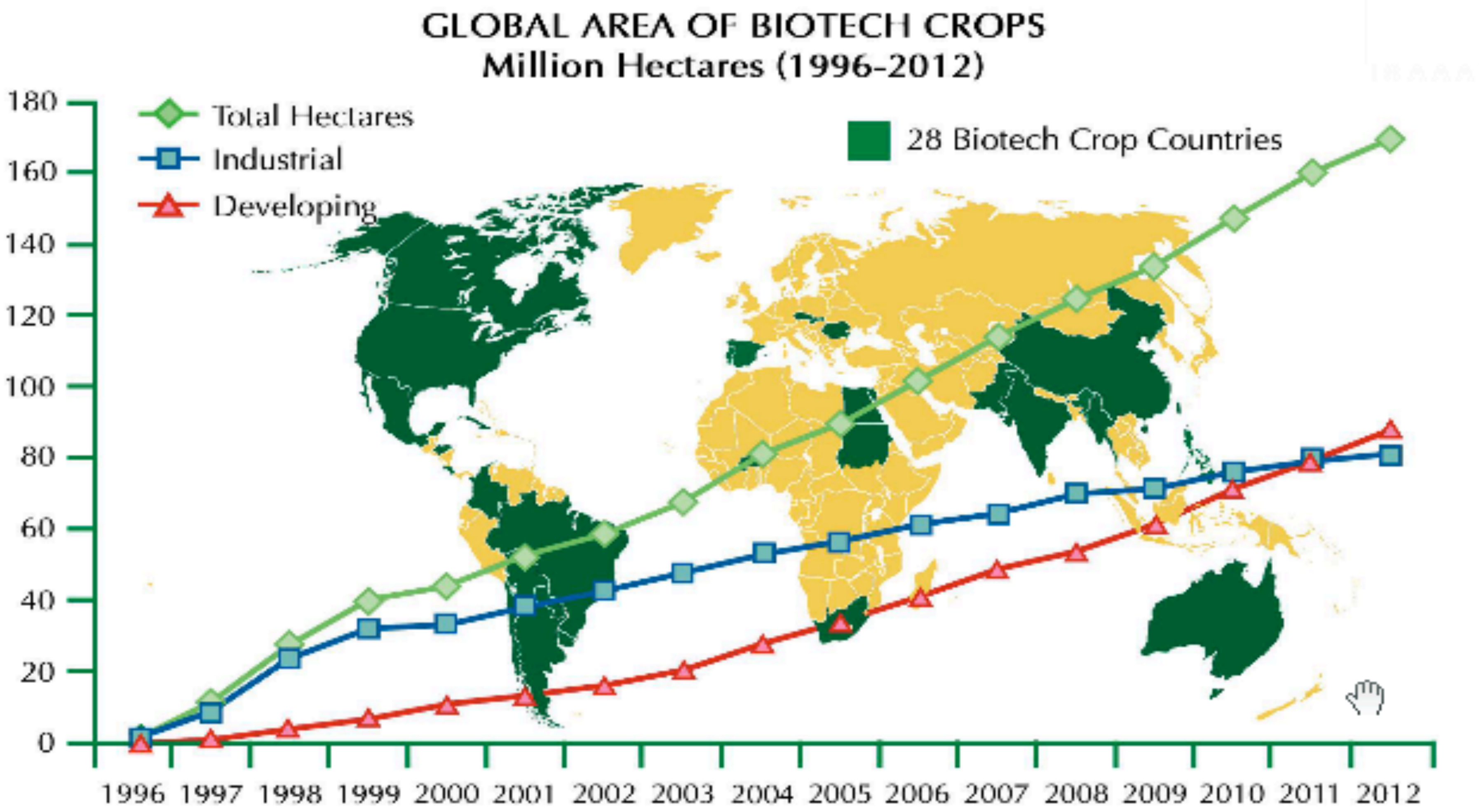
- It involves the insertion of DNA from one organism into another OR modification of an organism's DNA in order to achieve a desired trait.



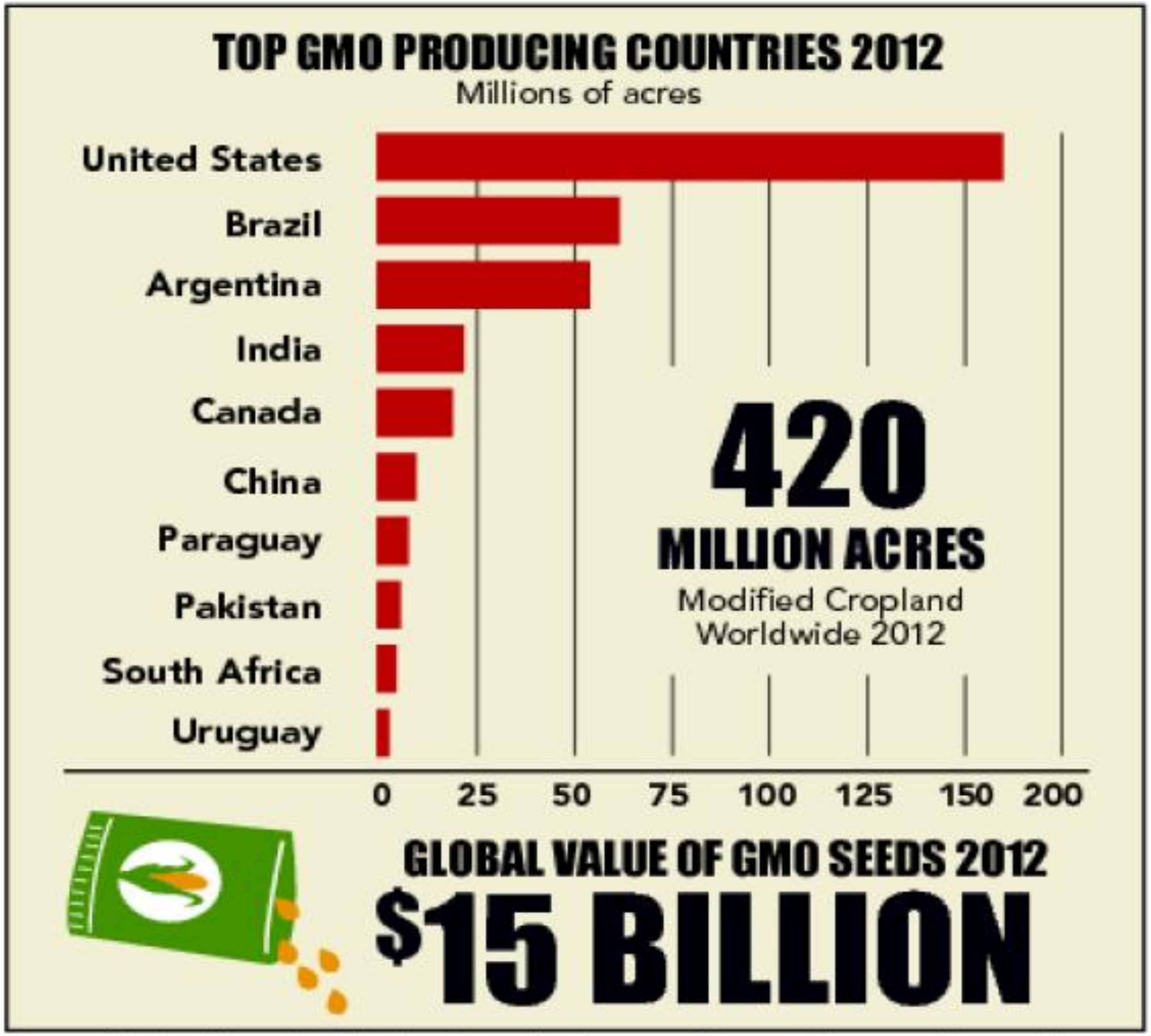
Suntory "blue" rose



Global status of commercial GM crops
2011, by millions of hectares
Canada
10.4m
Maize, soybean,
Canola, sugarbeet
US
69m
Maize, soybean, cotton,
Canola, sugarbeet, alfalfa,
papaya, squash
Mexico
0.2m
Cotton,
soybean
Honduras
<0.05m
Maize
Costa Rica
<0.05m
Cotton,
soybean
Colombia
<0.05m
Cotton
Brazil
30.3m
Maize, soybean,
cotton
Bolivia
0.9m
Soybean
Paraguay
2.8m
Soybean
Chile
<0.05m
Maize, soybean,
canola
Argentina
23.7m
Maize,
soybean,
cotton
Uruguay
1.3m
Soybean,
maize
Germany
<0.05m
Potato
Sweden
<0.05m
Potato
Czech Republic
<0.05m
Maize
Poland
<0.05m
Maize
Portugal
<0.05m
Maize
Spain
0.1m
Maize
Romania
<0.05m
Maize
Slovakia
<0.05m
Maize
Pakistan
2.6m
Maize
China
3.9m
Cotton, papaya, poplar,
tomato, sweet pepper
India
10.6m
Cotton
Burma
0.3m
Cotton
Egypt
<0.05m
Maize
Burkina Faso
0.3m
Cotton
Philippines
0.6m
Maize
South Africa
2.3m
Maize, soybean,
cotton
Australia
0.7m
Cotton, canola



A record 17.3 million farmers, in 28 countries, planted 170.3 million hectares (420 million acres) in 2012, a sustained increase of 6% or 10.3 million hectares (25 million acres) over 2011.

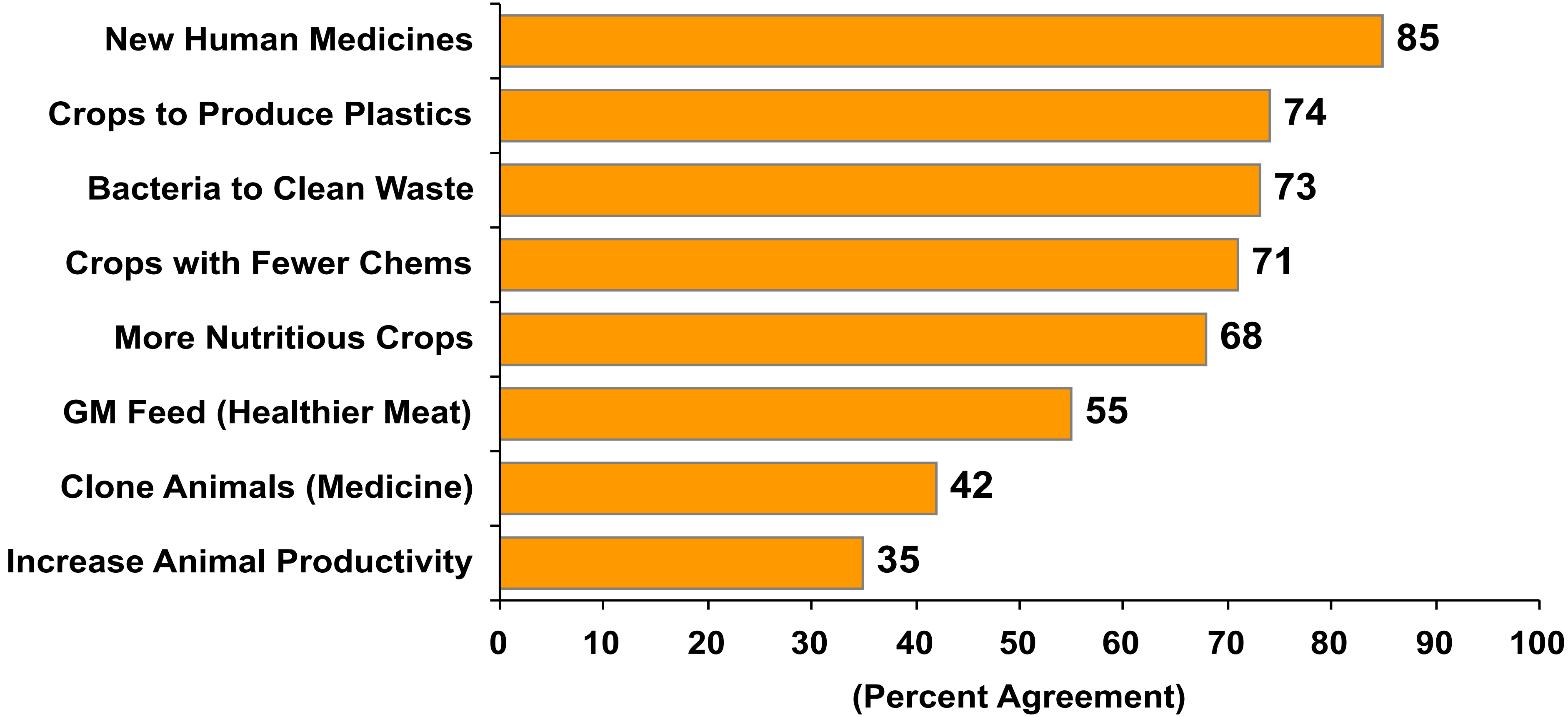


Source: Food and Drug Administration; International Service for the Acquisition of Agri-Biotech Applications

VALLEY NEWS — SHAWN BRALEY

Public Support Varies for Different Applications of Biotechnology

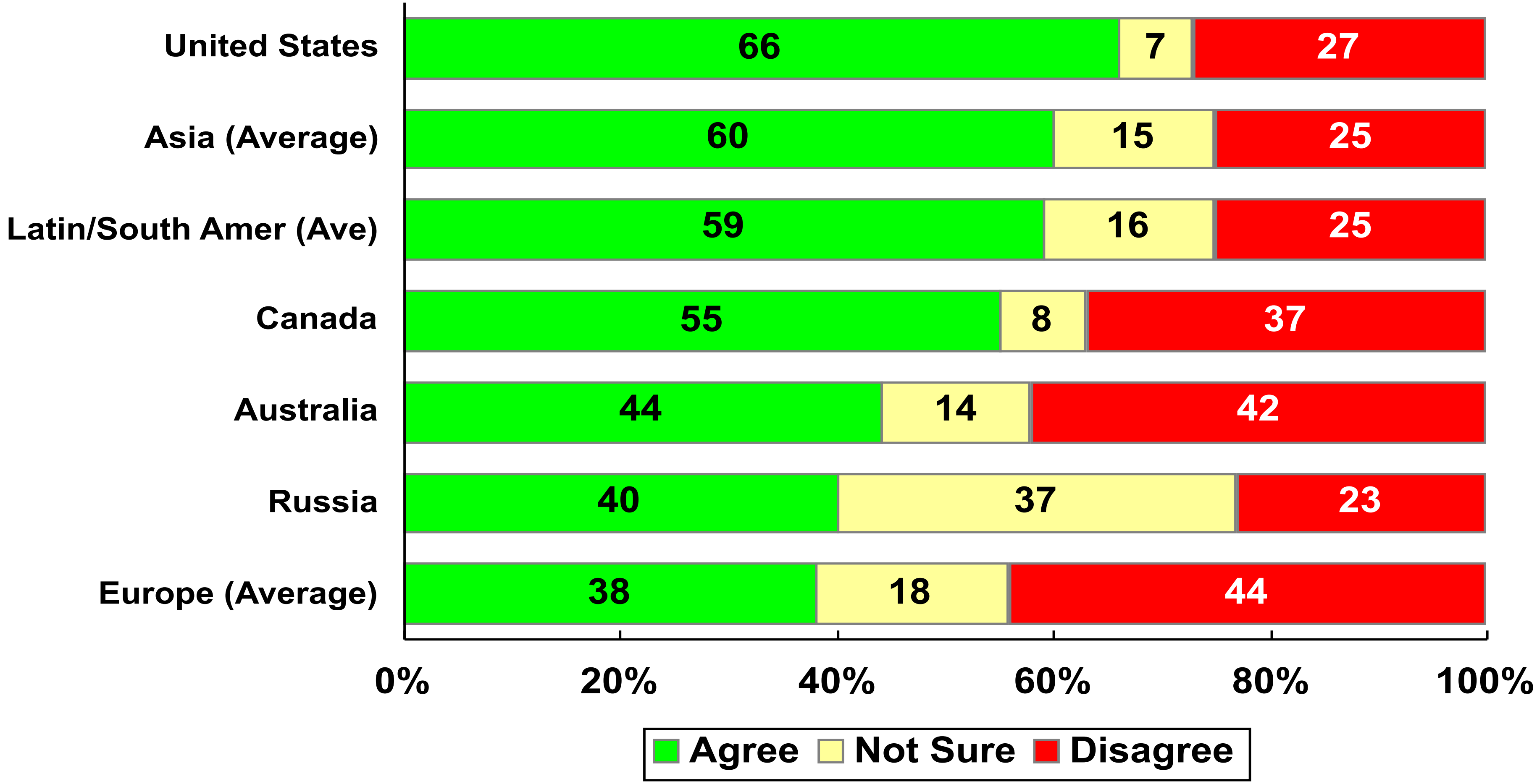
(Includes ALL Countries – N = 35,000)



Source: Thomas Hoban, North Carolina State University

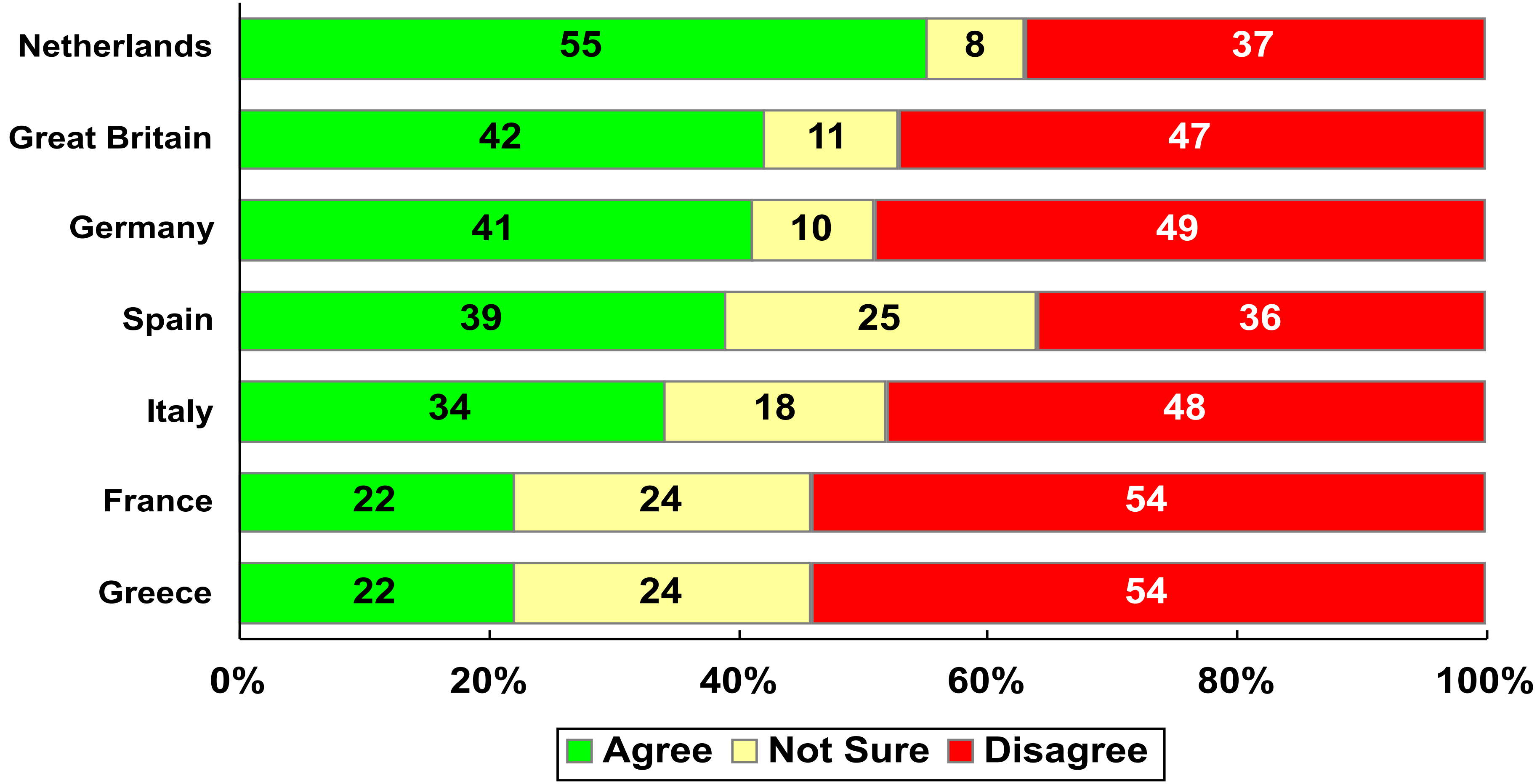
The Benefits of Using Biotechnology are Greater than the Risks

(35,000 Consumers from 35 Countries)

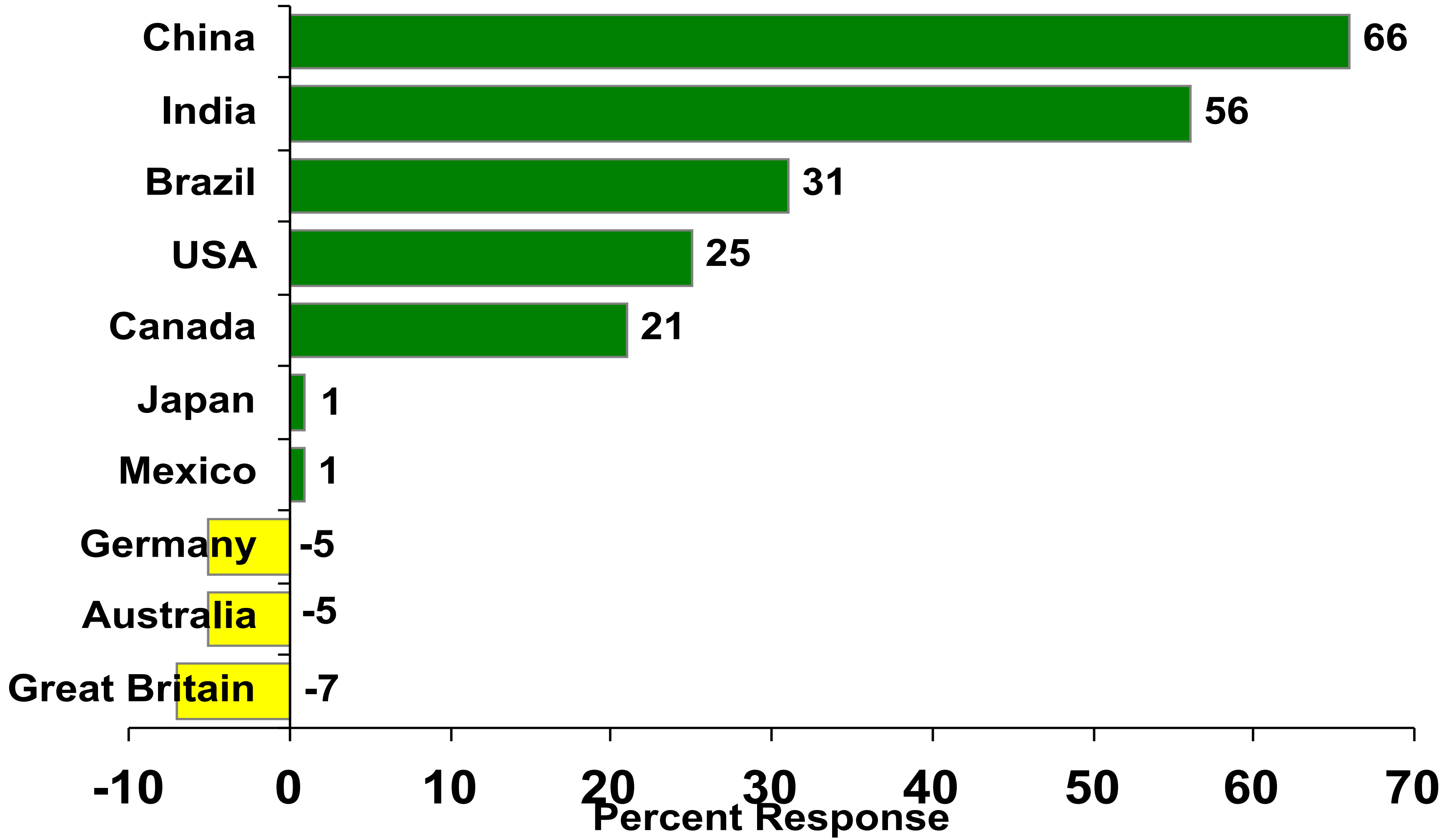


The Benefits of Using Biotechnology are Greater than the Risks

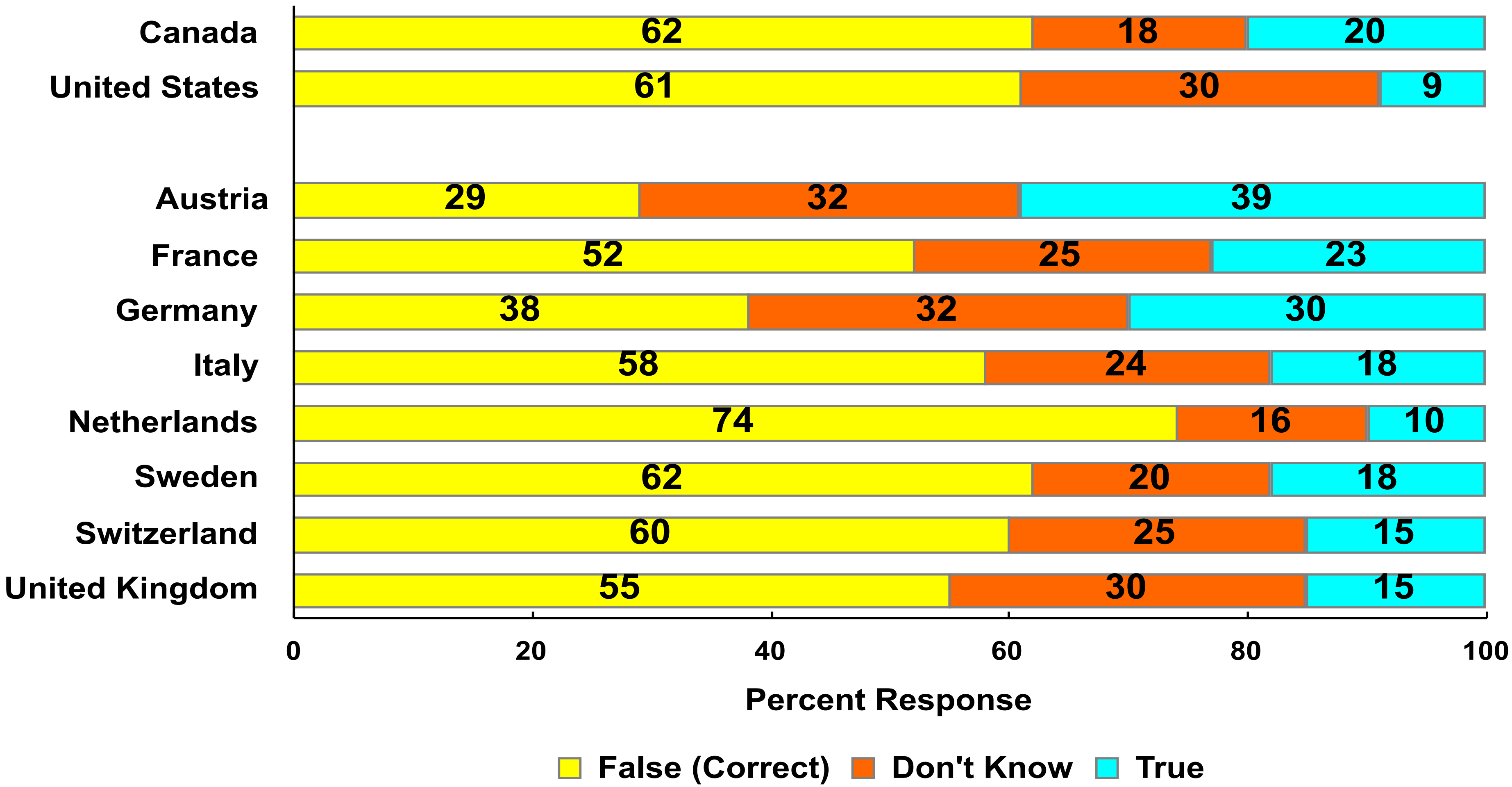
(European Consumers)



Willing to Buy GM Food if More Nutritious (Net = Continue – Not Continue)



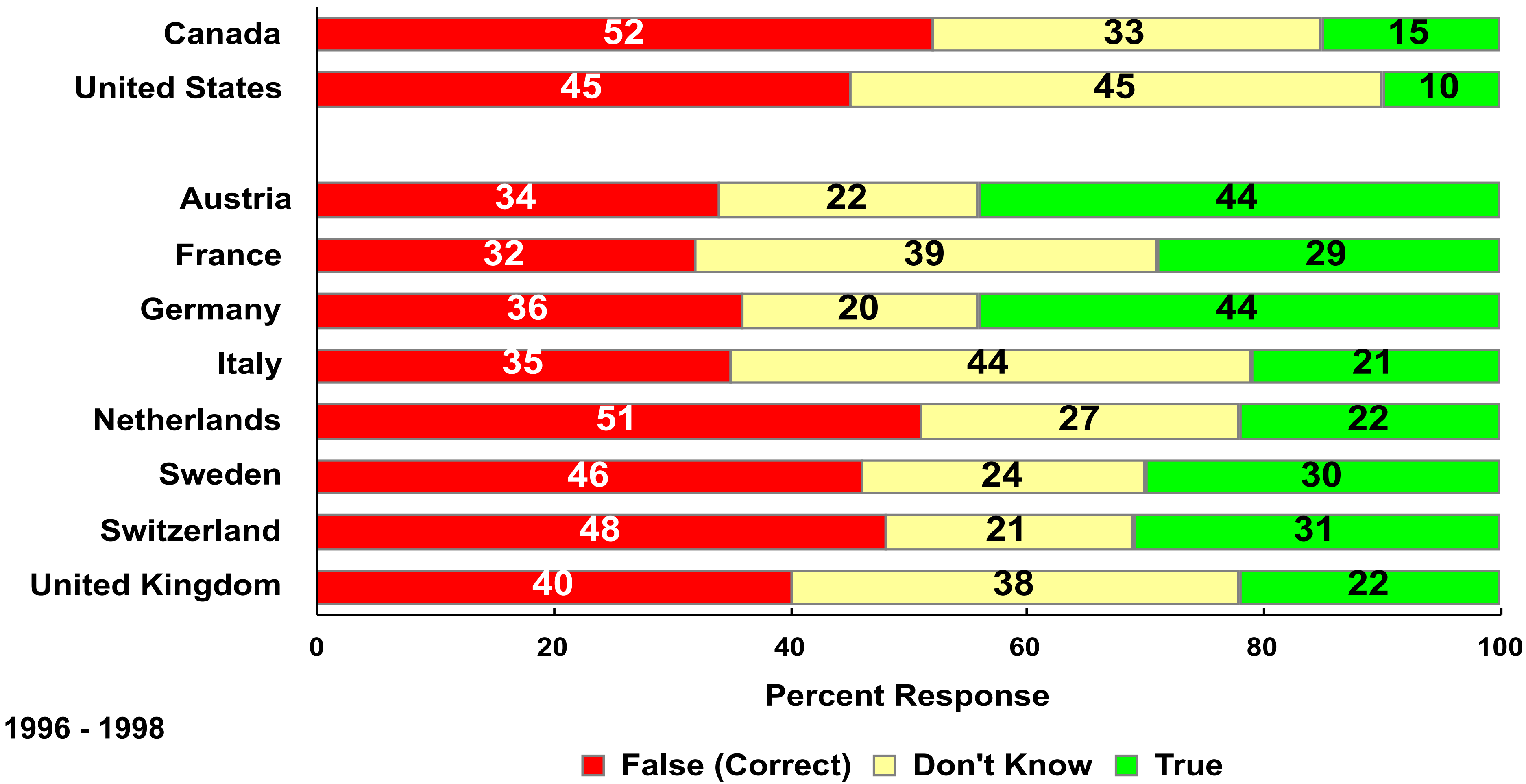
"By Eating a Genetically Modified Fruit, a Person's Genes Could also Be Changed"



1996 - 1998

Source: Thomas Hoban, North Carolina State University

"Ordinary Tomatoes Do Not Contain Genes, while Genetically Modified Ones Do"



A brief history of crop improvement

- **Strategies to manipulate genomes**
  - **Selection of desirable traits (manipulating population genetics)**
  - **Introducing new genetic traits to a genome**
    - **Gene transfer technologies**
    - **Genetic "transformation" (gene gun, *Agrobacterium tumefaciens*)**

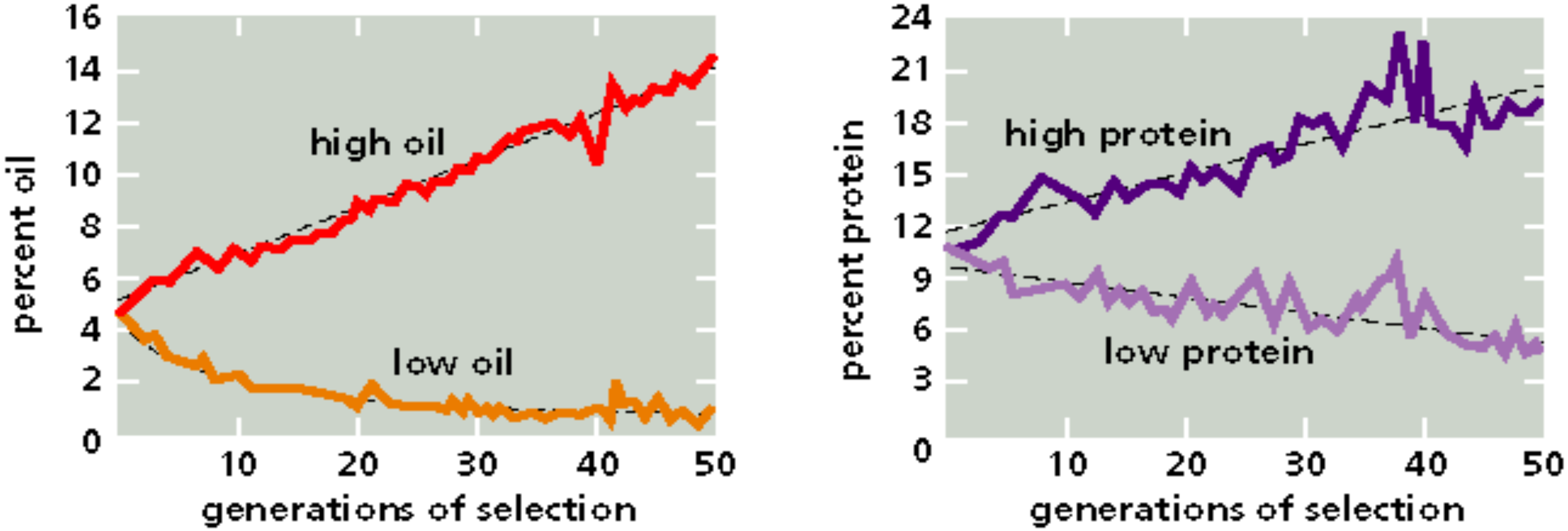
Crop domestication

- Artificial selection since ~9,000 B.C.

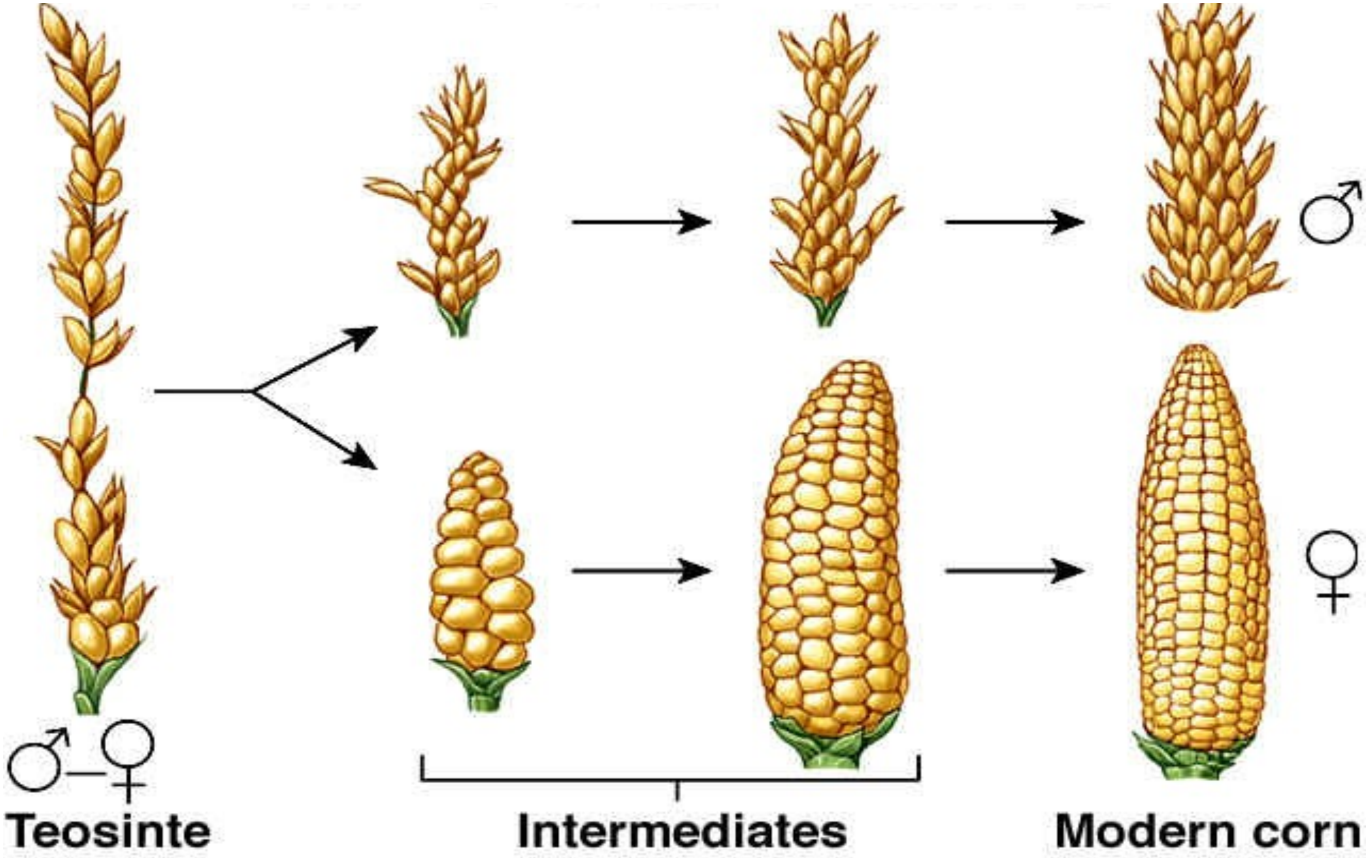
Introducing new traits: Hybridization

- Cross-pollination
  - Domesticated variety as one parent
  - Related variety or species with a desirable traits

Selection of desirable genetic traits



Agricultural breeding



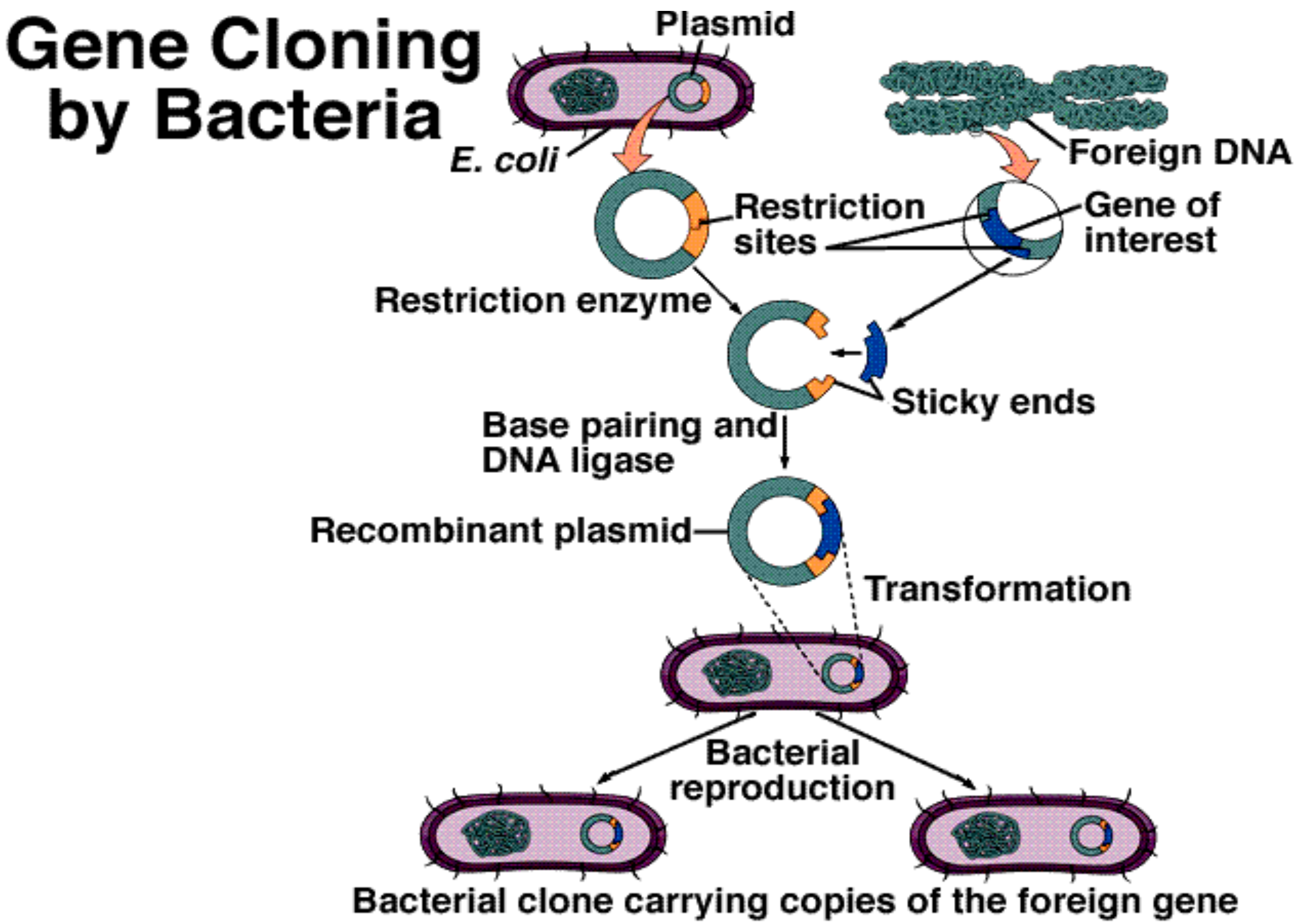
Traditional breeding changes organisms through selection.

Limitations of hybridization

- Restricted to plants that can naturally hybridize
  - Crosses to closely related species usually successful
  - Hybridization to distantly related species usually problematic
    - Little to no seed produced
    - Hybrids recovered are often sterile

Breaching reproductive barriers for crop improvement:

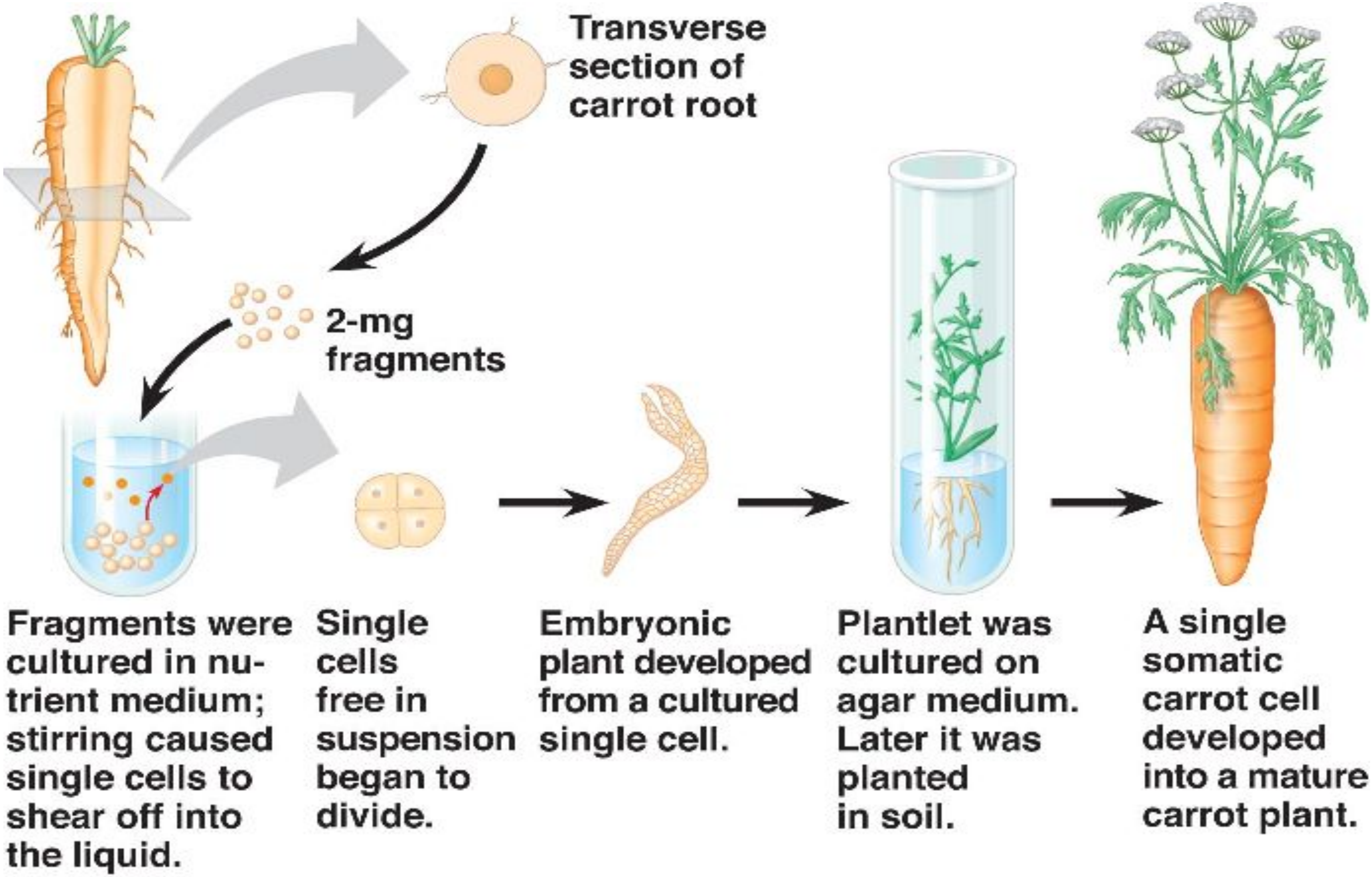
Recombinant DNA technology & genetic engineering



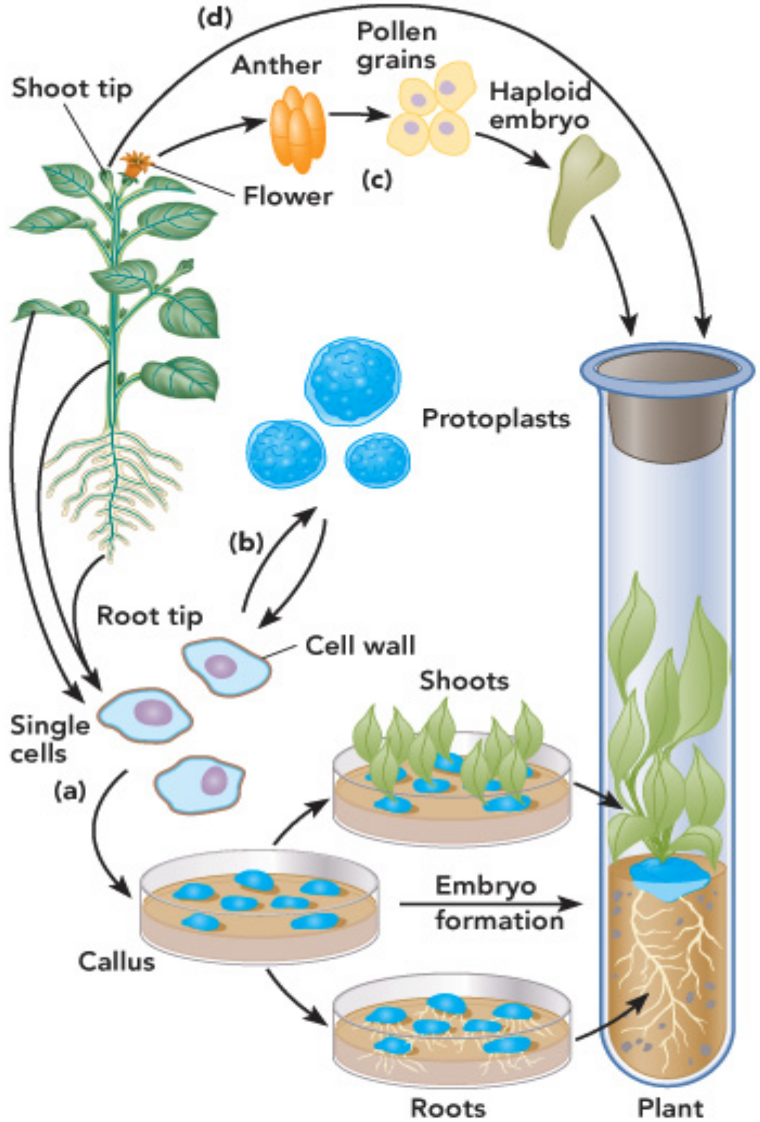
Recombinant DNA technology and crop improvement

- **Allows utilization of every species**
- **Allows direct transfer of a single gene**
- **Requires a method of gene transfer into plant cells**
- **Requires *regenerable* plant cells**
  - ***Totipotency* of many plant cell types allows for regeneration of entire plants from single cells**

TOTOPOTENCY OF PLANT CELLS



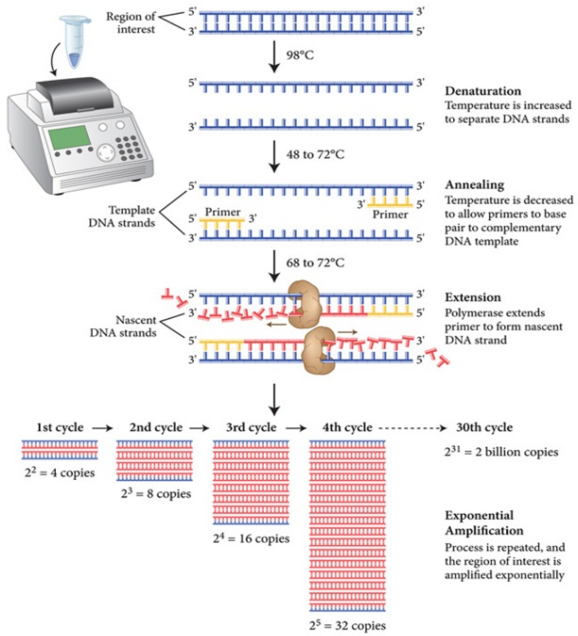
Whole plants can be regenerated via cell and tissue culture

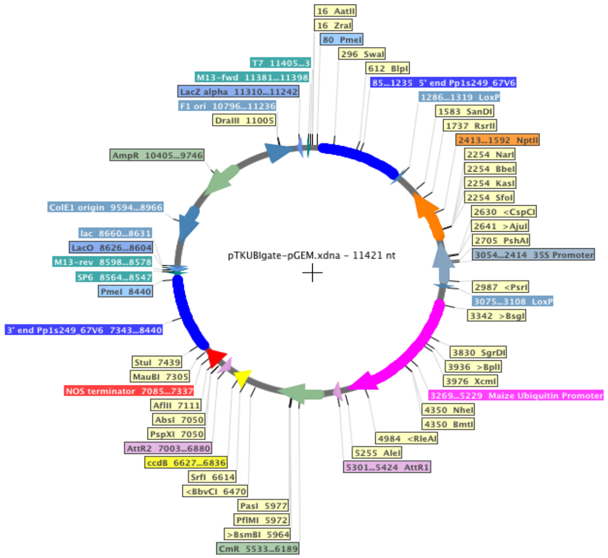


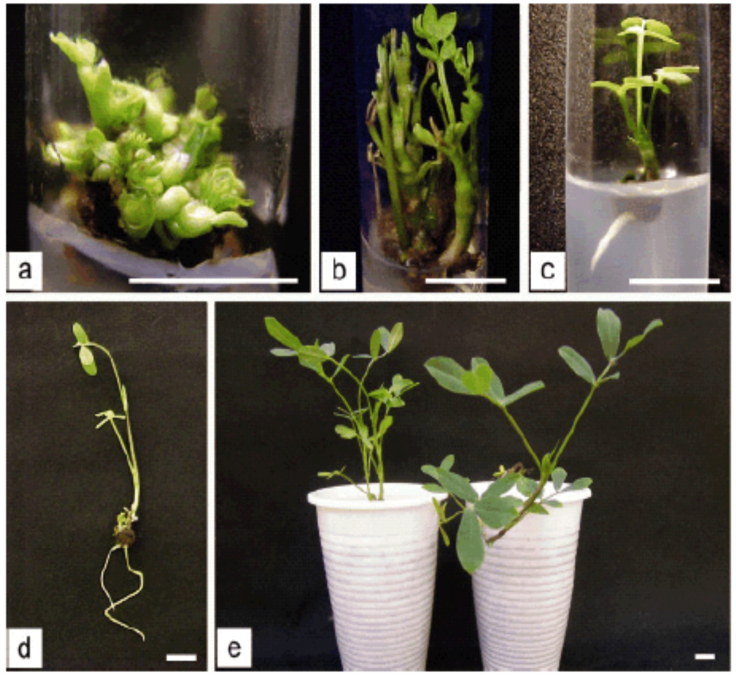
Plant transformation technologies

- **Genetic transformation – creating transgenic organisms**
  - **Agrobacterium**
  - **Gene gun**

General procedure







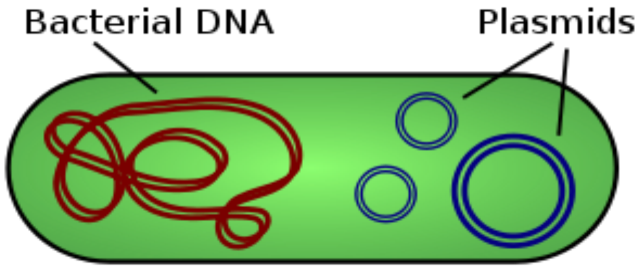
Gene cloning

Vector Construct

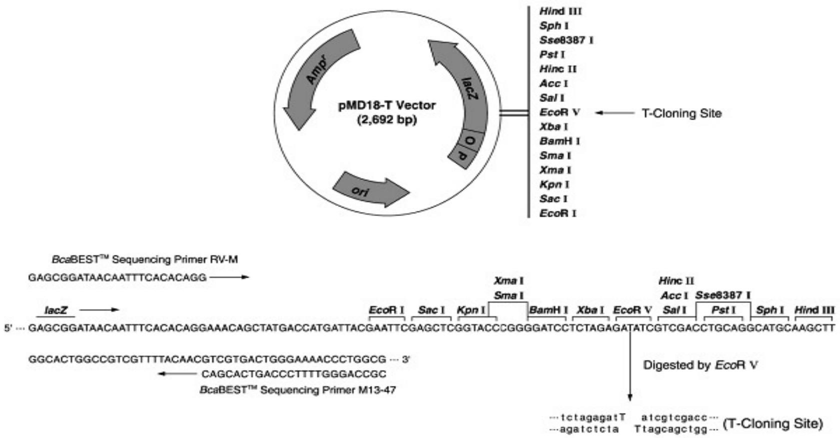
Plant transformation (Regeneration)

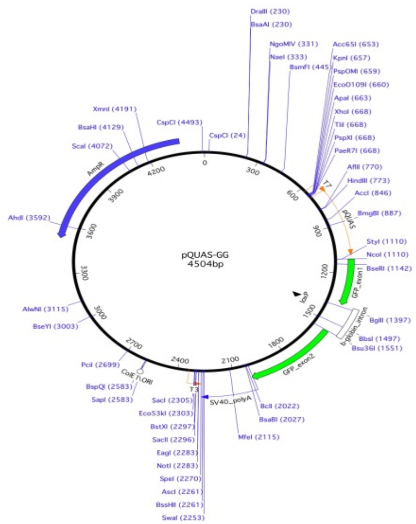
Vector Construct – Plasmid & Vector

Plasmid - small circular, double-stranded DNA molecules in bacteria, In nature, plasmids carry genes that may benefit survival of the organism.



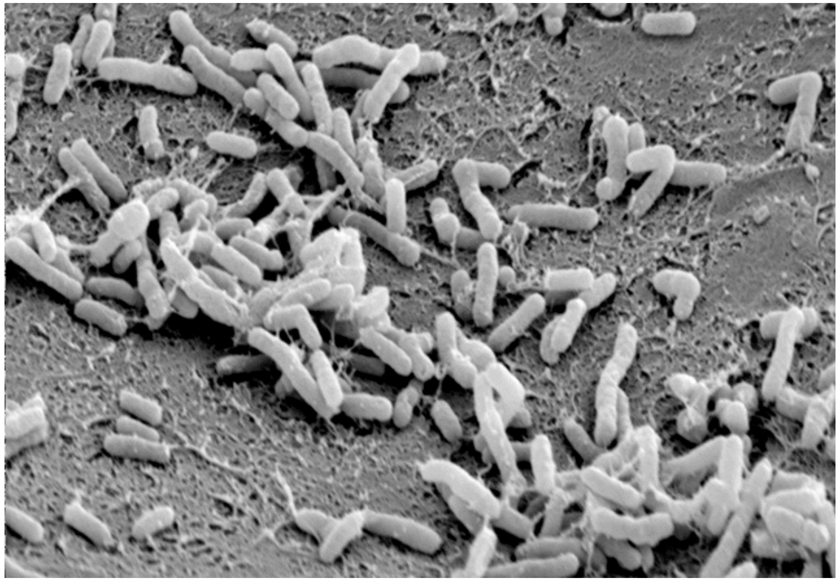
Vector - Plasmids used in genetic engineering are called vectors. They are commonly used to multiply (make many copies of) or express particular genes.

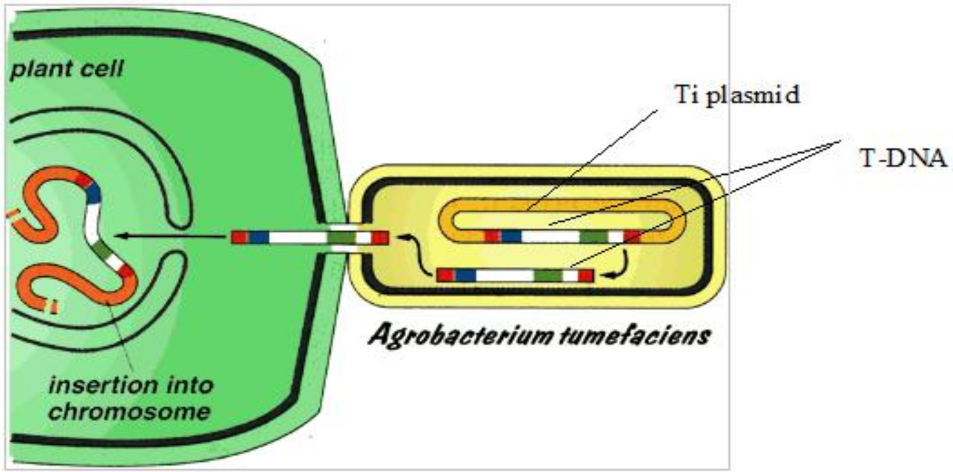




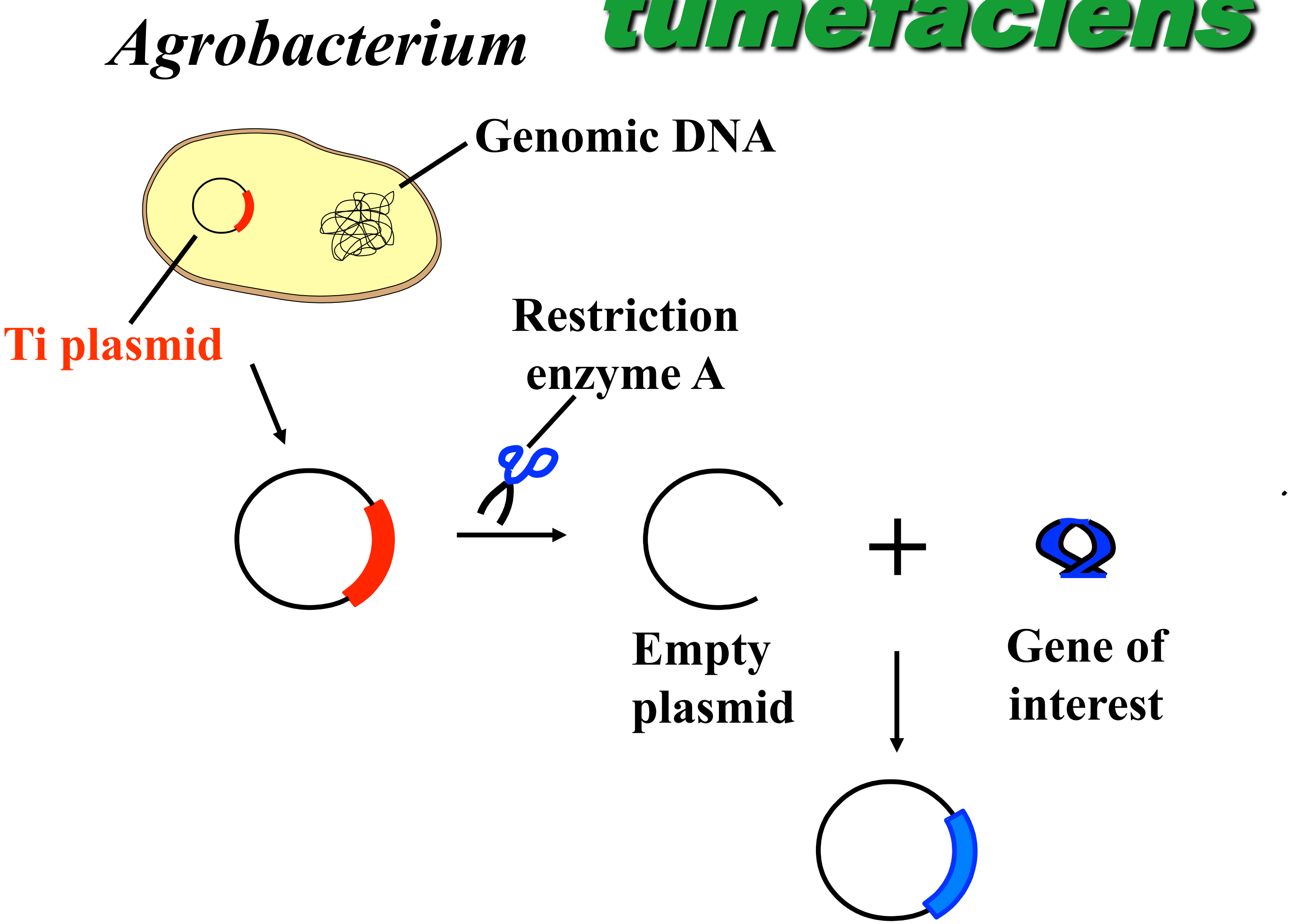
Vector Construct – *Agrobacterium tumefaciens*

Agrobacterium tumefaciens is the causal agent of **crown gall** disease (the formation of tumours) in over 140 species of dicot. It is a rod shaped, Gram negative soil bacterium. Symptoms are caused by the insertion of a small segment of DNA (known as the T-DNA, for 'transfer DNA'), from a plasmid, into the plant cell, which is incorporated at a semi-random location into the plant genome.



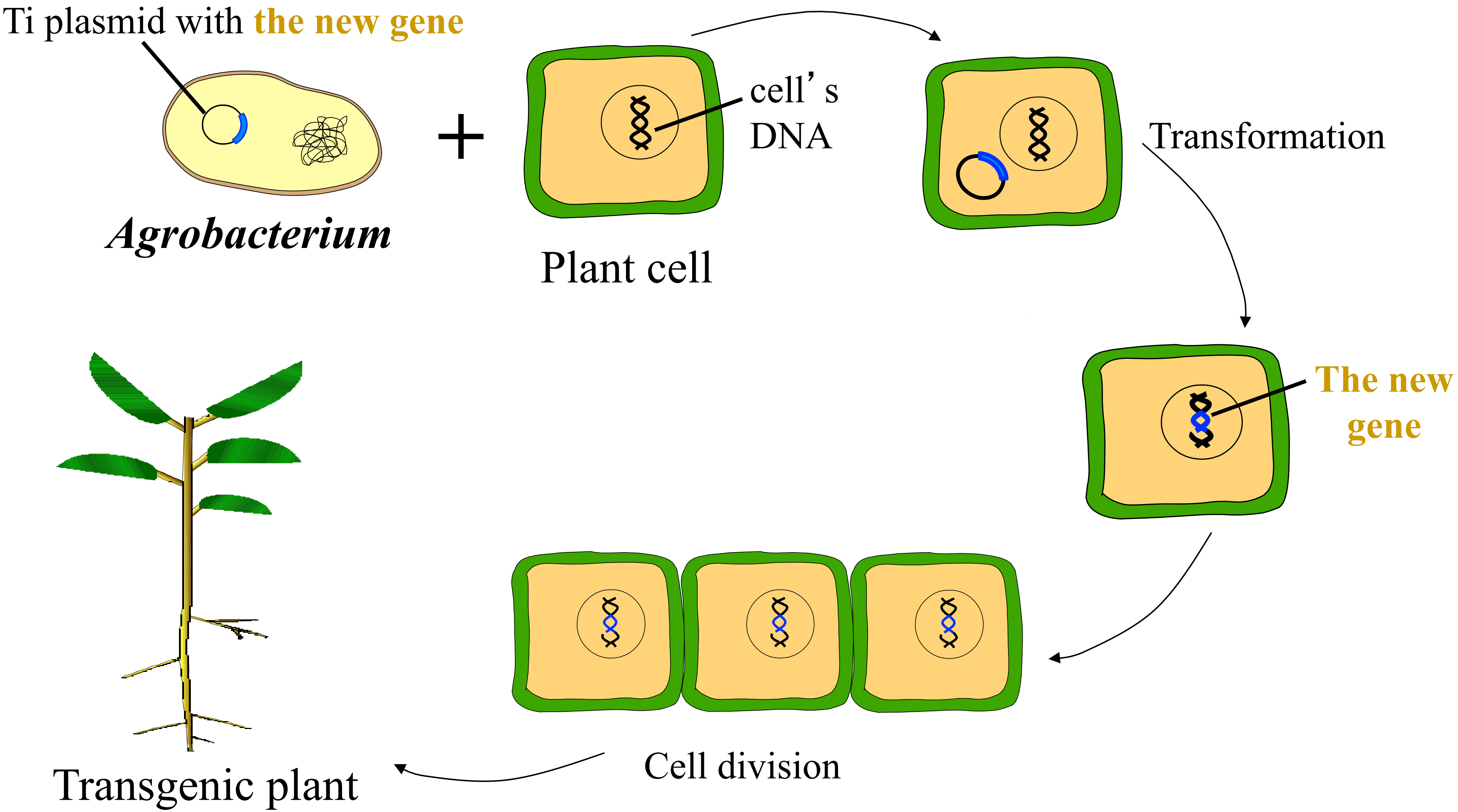


Agrobacterium tumefaciens

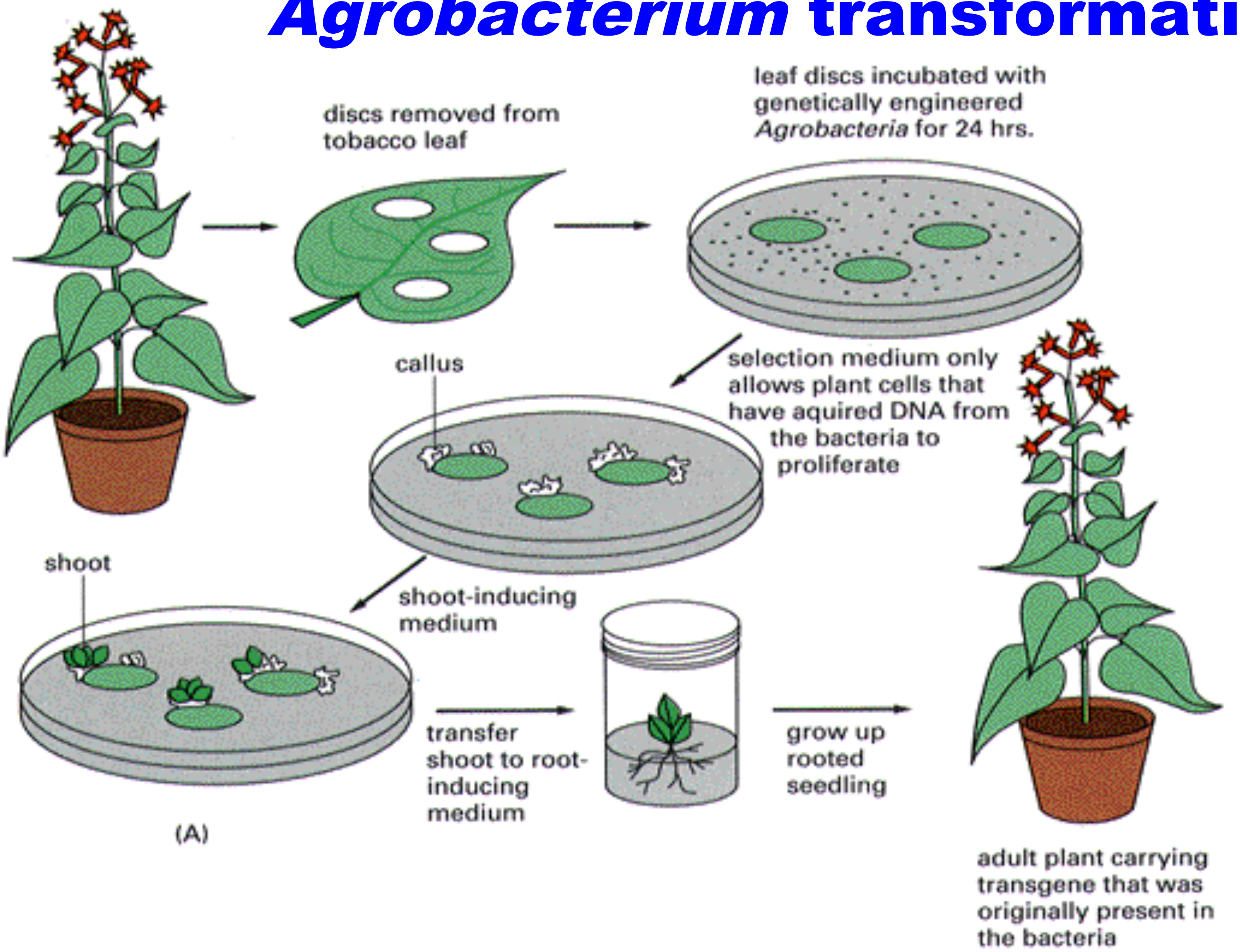


Ti plasmid with the gene of interest

Agrobacterium tumefaciens



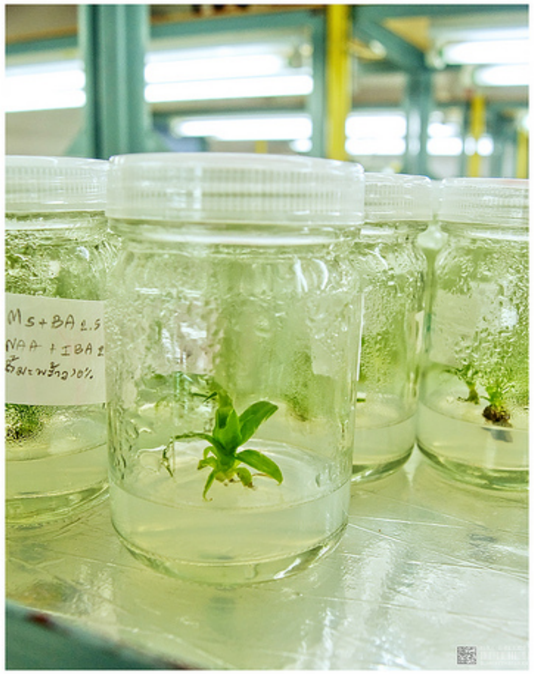
Agrobacterium transformation



Plant regeneration – plant tissue culture

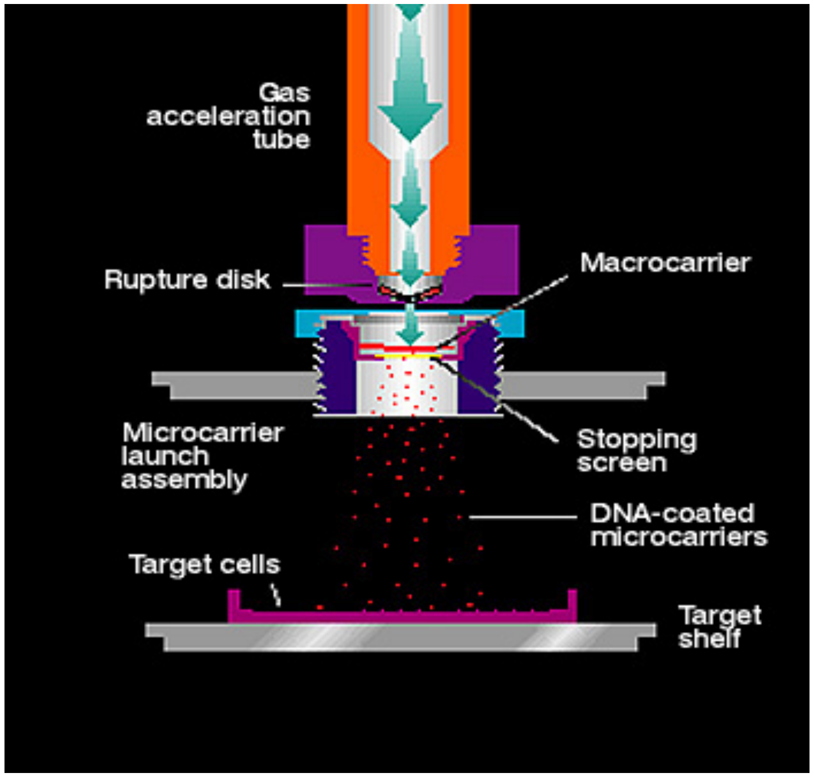
- **Plant tissue culture** is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition.

- Based on the development of the Plant Cell Totipotency.

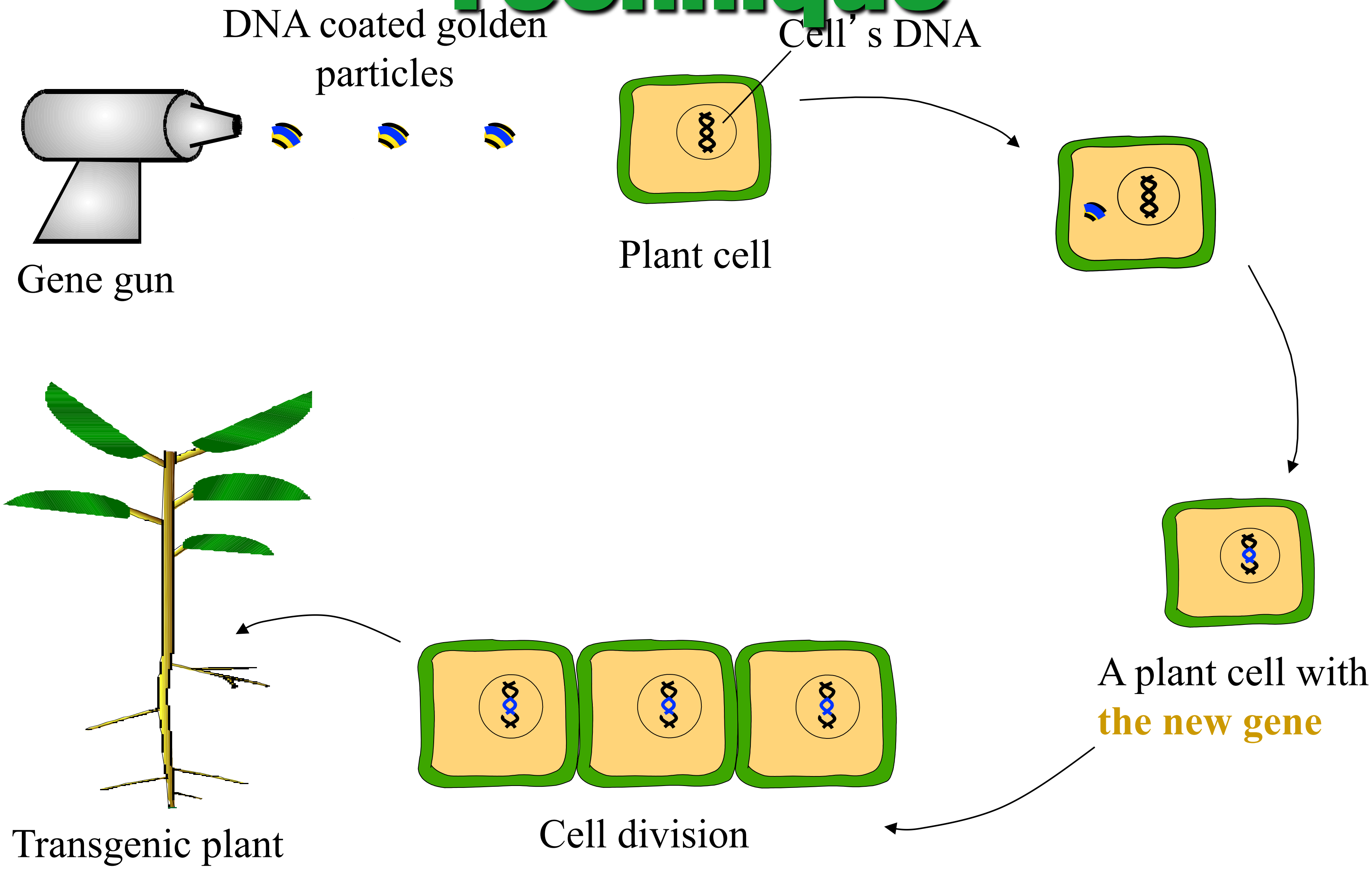


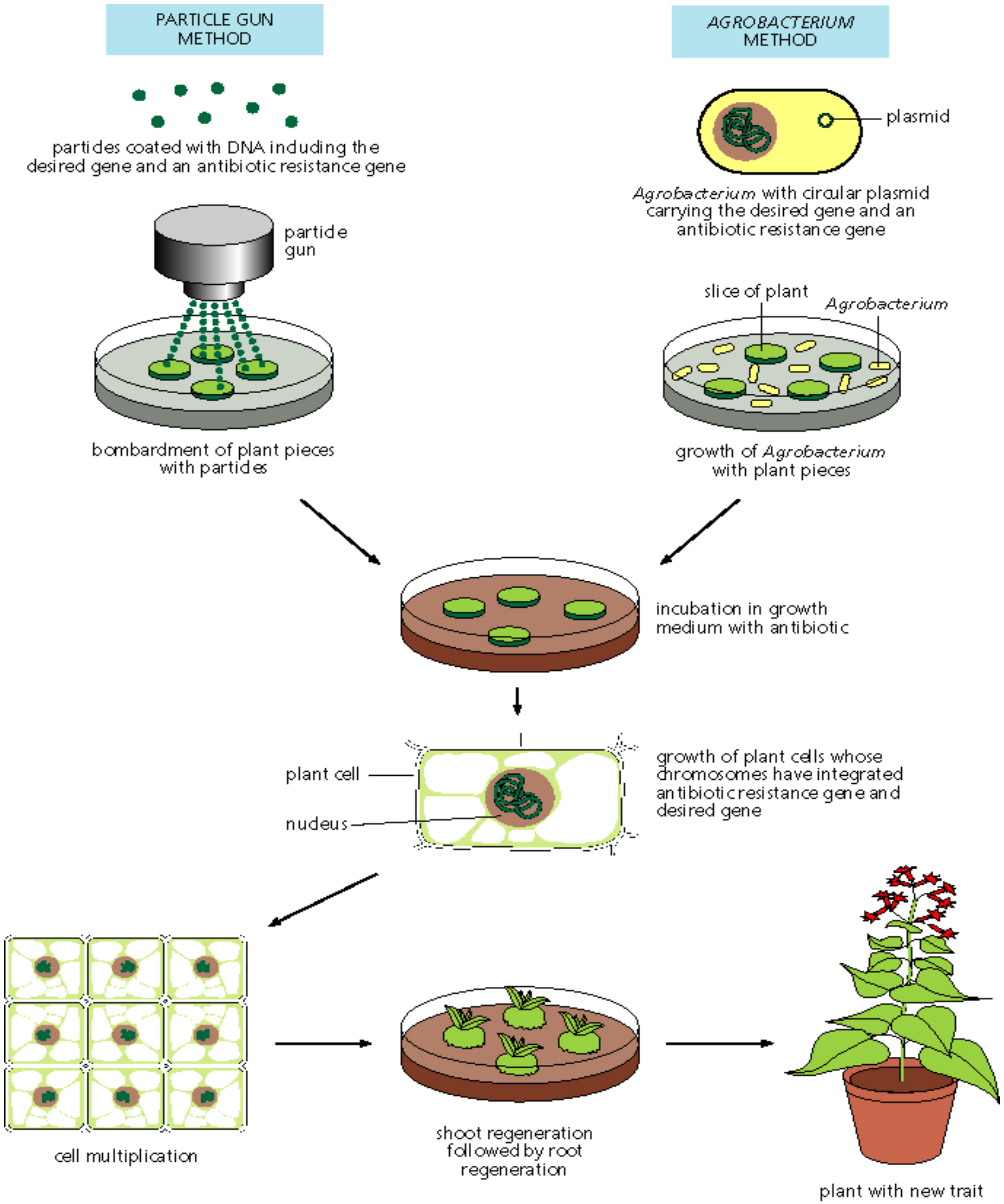
Gene Gun

- Invented by Cornell researcher, John Sanford



"Gene Gun" Technique





PARTICLE GUN METHOD
AGROBACTERIUM METHOD
particles coated with DNA including the desired gene and an antibiotic resistance gene
particle gun
bombardment of plant pieces with particles
plasmid
Agrobacterium with circular plasmid carrying the desired gene and an antibiotic resistance gene
slice of plant
Agrobacterium
growth of Agrobacterium with plant pieces
incubation in growth medium with antibiotic
plant cell
nucleus
growth of plant cells whose chromosomes have integrated antibiotic resistance gene and desired gene
cell multiplication
shoot regeneration followed by root regeneration
plant with new trait

Table 14.1 A Few Important Events in the Development of Plant Genetic Engineering

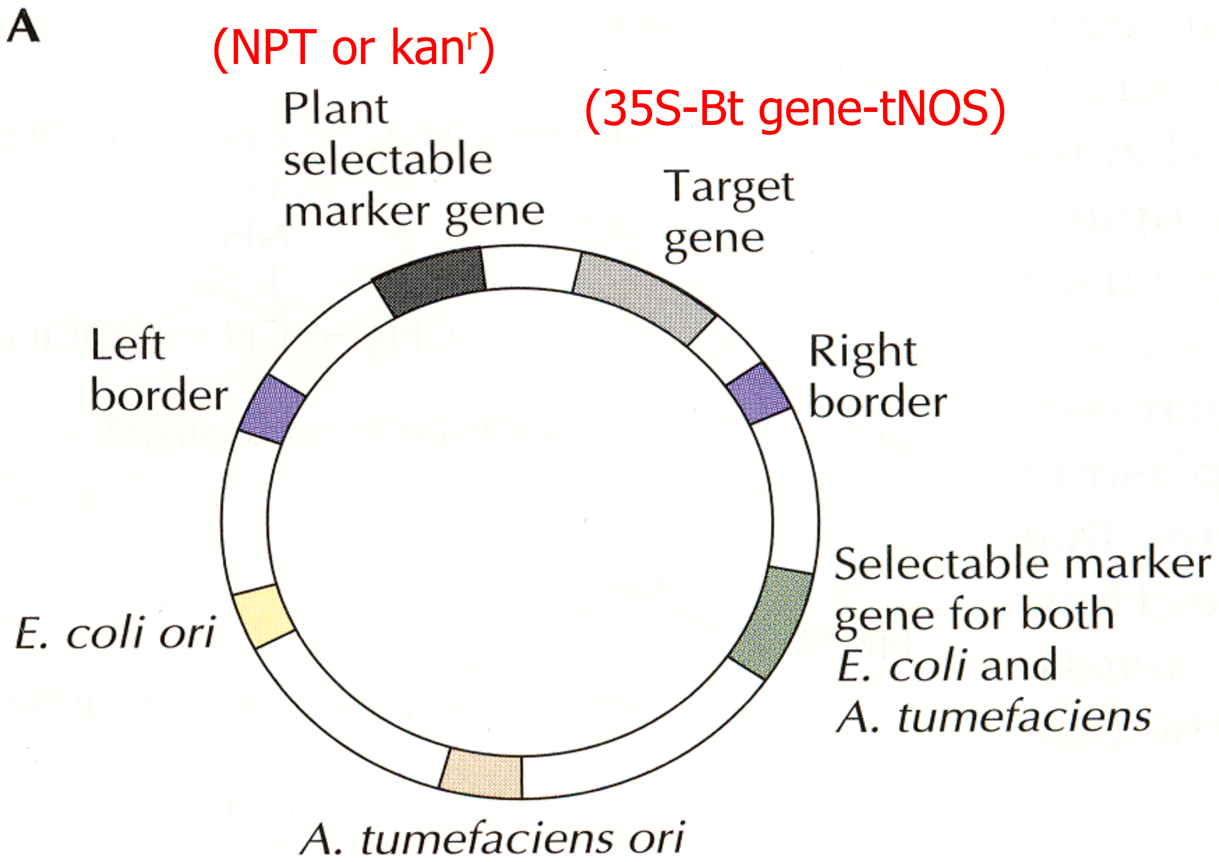
Year	Event
1866	Gregor Mendel determines the basic laws of heredity.
1882	Walther Fleming observes chromosomes.
1944	Oswald Avery, Colin MacLeod, and Maclyn McCarty prove that DNA is the genetic material.
1944	Frederick Sanger uses chromatography to determine the amino acid sequence of insulin.
1947	Gene transfer by plasmids is discovered.
1947	Barbara McClintock reports on transposons.
1953	James Watson and Francis Crick determine the structure of DNA.
1957	Francis Crick and George Gamov propose the central dogma of molecular biology, explaining how genes code for proteins.
1961	Marshall Nirenberg deciphers the first codon.
1964	Charles Yanofsky and colleagues prove that nucleotide sequences in DNA correspond to amino acid sequences in proteins.
1965	Restriction enzymes are discovered.
1969	The first gene is isolated from bacteria.
1972	Paul Berg uses restriction enzymes and DNA ligase to make the first recombinant DNA molecule.
1973	Stanley Cohen, Annie Chang, and Herbert Boyer make the first transgenic organism, a bacterium with a viral gene.
1976	The first genetic engineering company, Genentech, is founded in California.
1977	Frederick Sanger announces his chain termination method of DNA sequencing.
1978	Genentech and the City of Hope National Medical Center announce the lab production of a gene for human insulin.
1980	Human insulin becomes the first useful product made by transgenic bacteria.
1980	The first patent is issued for genetically engineered bacteria.
1983	Eli Lilly receives a license to make human insulin.
1985	Kary Mullis develops the polymerase chain reaction.
1985	The first automated DNA sequencer is invented.
1985	Disease-resistant transgenic plants are field-tested for the first time.
1985	The first genetically engineered crop, a tobacco, is approved for release by the EPA.
1986	The first field test is carried out on a genetically engineered plant.
1987	Genetic engineering patents are applied to plants and animals.
1987	Advanced Genetic Sciences conducts a field trial of bacteria to prevent frost formation on strawberries.
1987	Calgene receives a patent to extend the shelf life of tomatoes by producing antisense RNA that silences the polygalacturonase gene.
1990	Transformation of corn with a gene gun is announced.
1994	Calgene wins FDA approval for the Flavr Savr® tomato.
2000	The genome of *Arabidopsis thaliana* is completely sequenced.
2002	The genome of rice (*Oryza sativa*) is completely sequenced.

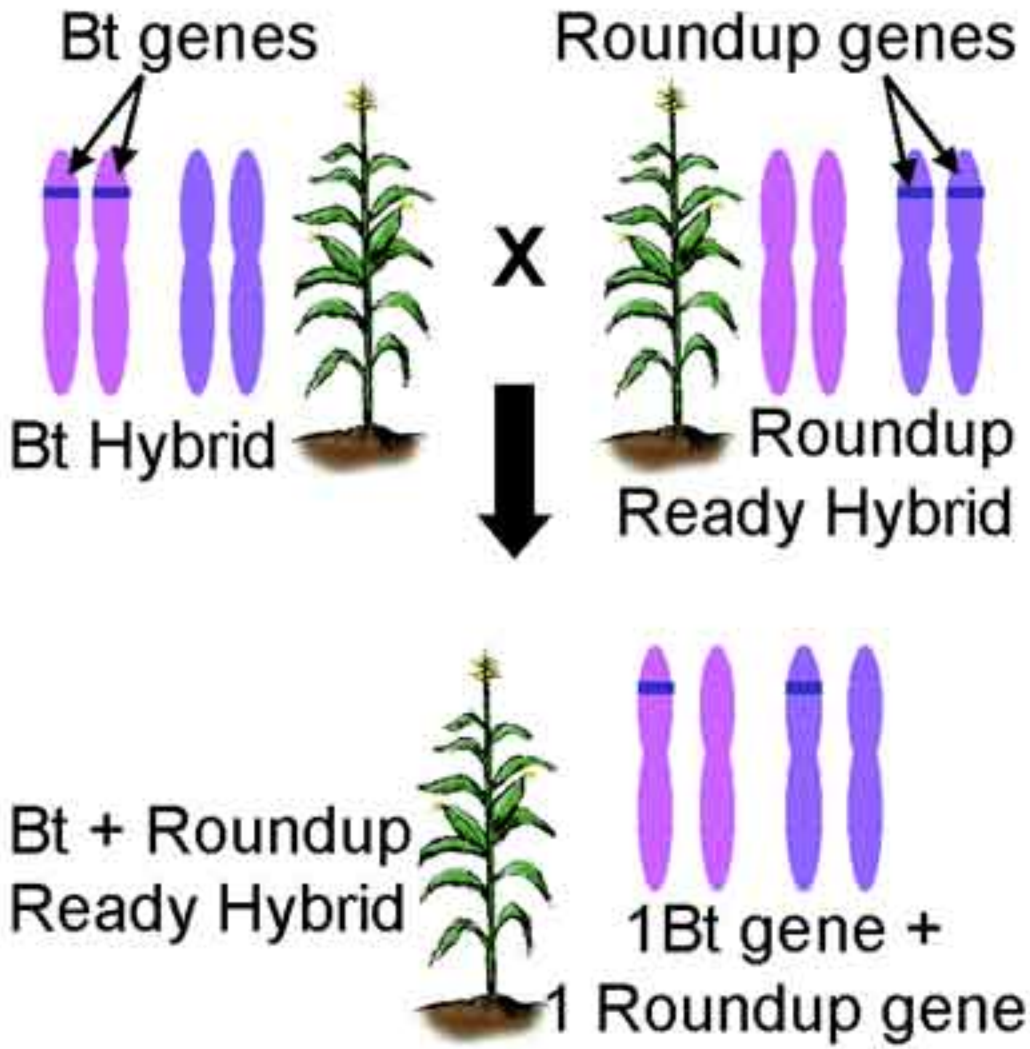
Examples of crop improvement through genetic engineering

- **Engineered herbicide resistance**
  - **Glyphosate (RoundUp™) and EPSP synthase**

A binary T-DNA plasmid for delivering the Bt gene to plants

Bacillus thuringiensis, commonly known as Bt, is a bacterium that occurs naturally in the soil. Some strains of Bt produce proteins that kill certain insects. When these insects ingest the Bt protein, the function of their digestive systems is disrupted, producing slow growth and, ultimately, death




Bt genes
Roundup genes
Bt Hybrid
X
Roundup
Ready Hybrid
Bt + Roundup
Ready Hybrid
1Bt gene +
1 Roundup gene

Genetically engineered Bt-plants in the field

Product	Institution(s)	Engineered Trait(s)	Sources of New Genes	Name
Corn	Bayer	Resist glufosinate herbicide to control weeds/Bt toxin to control insect pests (European corn borer)	Bacteria, virus	StarLink-1998 (animals only)
Corn	Dow/Mycogen	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	NatureGard-1995
Corn	Dow/Mycogen DuPont/Pioneer	Resist glufosinate herbicide to control weeds/Bt toxin to control insect pests (Lepidopteran)	Corn, bacteria, virus	Herculex I-2001
Corn	Monsanto/DeKalb	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt-Xtra-1997
Corn	Monsanto	Bt toxin to control insect pests (European corn borer)	Bacteria	YieldGard-1996
Corn	Monsanto	Resist glyphosate herbicide to control weeds/Bt toxin to control insect pests (European corn borer)	Arabidopsis, bacteria, virus	?-1998
Corn	Syngenta	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt11-1996
Corn	Syngenta	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	Knock Out-1995
Corn (pop)	Syngenta	Bt toxin to control insect pests (European corn borer)	Corn, bacteria, virus	Knock Out-1998
Corn (sweet)	Syngenta	Bt toxin to control insect pests (European corn borer)	Bacteria	Bt11-1998
Cotton	Monsanto/Bayer	Resist bromoxynil herbicide to control weeds/Bt toxin to control insect pests (cotton bollworms	Bacteria	?-1998
		and tobacco budworm)		
Cotton	Monsanto	Bt toxin to control insect pests (cotton bollworms and tobacco budworm)	Bacteria	Bollgard-1995
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)	Bacteria	NewLeaf-1995
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)/resist potato virus Y	Bacteria, virus	NewLeaf Y-1999
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)/resist potato leafroll virus	Bacteria, virus	NewLeaf Plus-1998

GENETIC TRAITS EXPRESSED IN GMOs IN THE U.S.

RAINBOW PAPAYA

Genetic Traits

Disease resistance

Uses

- Table fruit

FIELD CORN

Genetic Traits

Insect Resistance
Herbicide Tolerance

Uses

- Livestock and poultry feed
- Fuel ethanol
- High-fructose corn syrup and other sweeteners
- Corn oil
- Starch
- Cereal and other food ingredients
- Alcohol
- Industrial uses

CANOLA

Genetic Traits

Herbicide Tolerance

Uses

- Cooking oil
- Animal feed

SOYBEAN

Genetic Traits

Insect Resistance Herbicide Tolerance

Uses

- Livestock and poultry feed
- Aquaculture
- Soybean oil (vegetable oil)
- High oleic acid (monounsaturated fatty acid)
- Biodiesel fuel
- Soymilk, soy sauce, tofu, other food uses
- Lecithin
- Pet food
- Adhesives and building materials
- Printing ink
- Other industrial uses

ALFALFA

Genetic Traits

Herbicide Tolerance

Uses

- Animal feed

COTTON

Genetic Traits

Insect Resistance
Herbicide Tolerance

Uses: Fiber, Animal feed, Cottonseed oil

SUGAR BEETS

Genetic Traits

Herbicide Tolerance

Uses: Sugar, Animal feed

SWEET CORN

Genetic Traits

Insect Resistance
Herbicide Tolerance

Uses: Food

SUMMER SQUASH

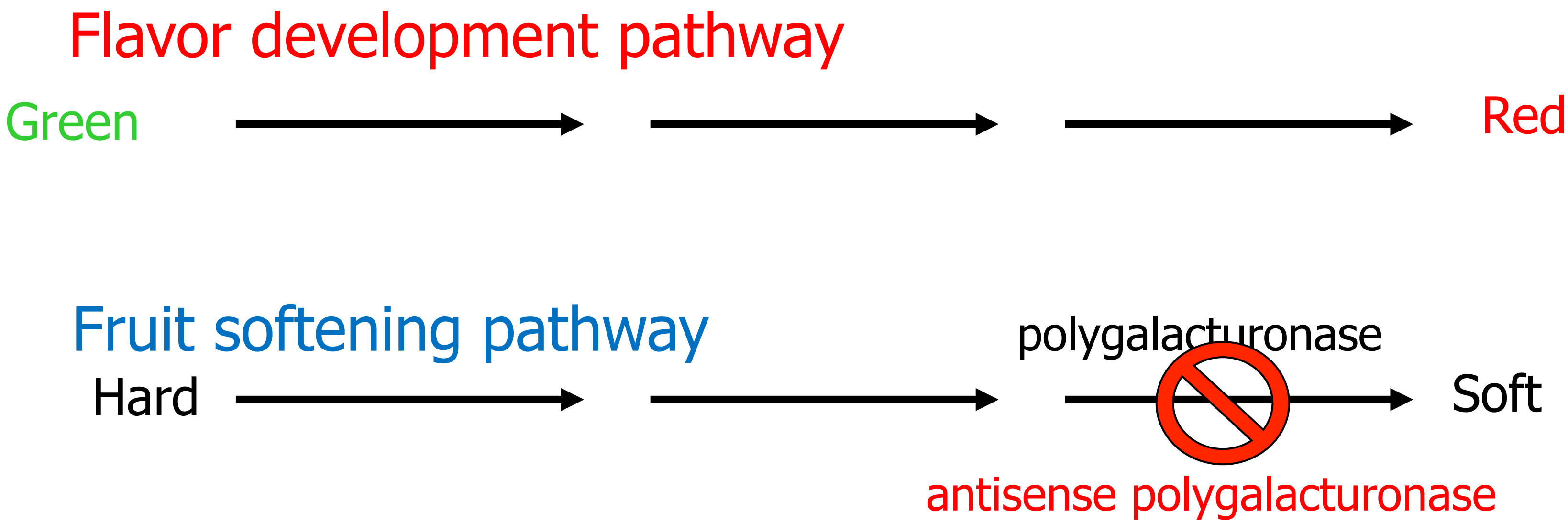
Genetic Traits

Disease resistance

Uses: Food

Development of stress- and senescence-tolerant plants: genetic engineering of flavorful tomatoes

- Fruit ripening is a natural aging or senescence process that involves two independent pathways, flavor development and fruit softening.
- Typically, tomatoes are picked when they are not very ripe (i.e., hard and green) to allow for safe shipping of the fruit.
- Polygalacturonase is a plant enzyme that degrades pectins in plant cell walls and contribute to fruit softening.
- In order to allow tomatoes to ripen on the vine and still be hard enough for safe shipping of the fruit, polygalacturonase gene expression was inhibited by introduction of an antisense polygalacturonase gene and created the first commercial genetically engineered plant called the FLAVR SAVR tomato.



Some genetically modified foods

Golden rice

Transgenic technology produced a type of rice that accumulates beta-carotene in rice grains. Once inside the body, beta-carotene is converted to vitamin A.

More than 120 million children in the world suffers from vitamin A deficiency. Golden Rice has the potential to help prevent the 1 to 2 million deaths each year caused by a deficiency in this vitamin.

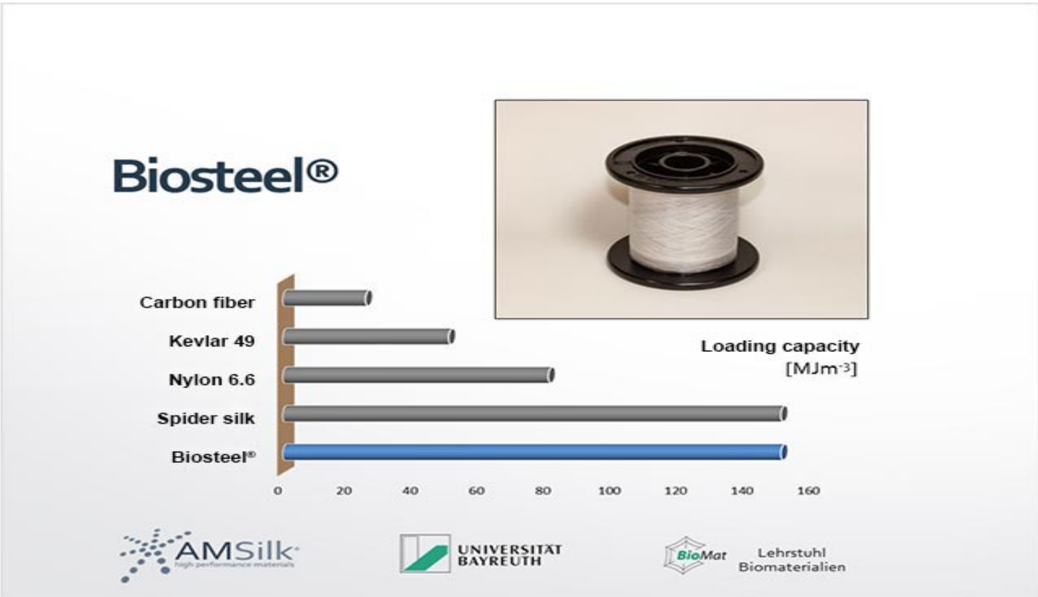
Some genetically modified foods

AquAdvantage salmon

GMO in Manufacturing

+

- Produces silk in milk to make Biosteel



Plants as bioreactors

- **Production of therapeutic agents (proteins)**
- **Production of recombinant vaccines or edible vaccines**
- **Production of antibodies**

- **Molecular farming !!!!**

Table 20.6

TABLE 20.6 Comparison of recombinant protein production in plants and other systems

Parameter	Bacteria	Yeast	Mammalian cell culture	Transgenic plants
Glycosylation	None	Incorrect	Correct	Generally correct
Assembles multimeric proteins	Limited	Limited	Limited	Yes
Production costs	Medium	Medium	High	Low
Protein-folding accuracy	Low	Medium	High	High
Protein yield	High	High	Medium	Medium
Scale-up costs	High	High	High	Low
Time required	Low	Low	High	Medium
Skill level required for growth	Medium	Medium	High	Low

PLANT-DERIVED BIOMEDICALS FOR TREATMENT OF INFECTIOUS DISEASES

DISEASES	VACCINES	PLANT	ANTIBODY	PLANT
Respiratory Syncytial	⬋	Tobacco		
Hepatitis B	⬋	Lettuce		
HIV	⬋	Spinach		
Rabies	⬋	Spinach Tobacco	⬋	Tobacco
Anthrax	⬋	Tobacco Tomato		
Diphtheria	⬋	Tobacco		
SARS	⬋	Tobacco Tomato		
Smallpox	⬋	Tobacco Tomato		

PLANT-DERIVED BIOMEDICALS FOR TREATMENT OF CANCER

HUMAN DISEASES	VACCINES	ANTIBODY
Colorectal Cancer	⬋	⬋
Epithelial Tumors (EGF receptor)		⬋

Antibodies

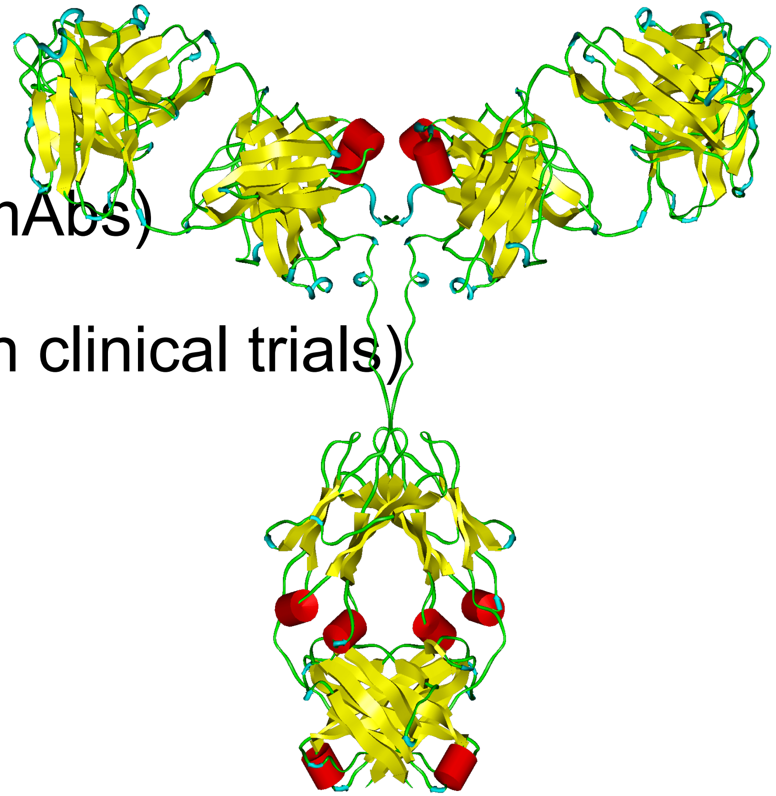
- **Magic bullets:**
- **Therapeutic – inhibit target involved in disease progression**
- **Diagnostic**
- **Prevention of disease**
- **Cause cytotoxic death of target cells**
- **Act as carriers for radioisotopes, toxins and drugs to site of disease**

Full-size monoclonal antibodies recently produced in transgenic plants

Plant	Antibody type (target)	Purpose
Tobacco	IgG (low molecular weight phosphonate ester)	Catalytic antibodies
Tobacco	IgG (nematode)	Plant pathogen resistance
Tobacco	sIgA/G (*Streptococcus mutans*)	Therapeutic (topical)
Soybean, rice	IgG (herpes simplex virus)	Therapeutic (topical)
Tobacco	IgG (colon cancer)	Therapeutic (systemic injection)
Alfalfa	IgG (human IgG)	Diagnostic
Tobacco	IgG (rabies virus)	Therapeutic: first IgG expressed in plant showing therapeutic activity (systemic injection)
Tobacco	IgG (hepatitis B virus)	Immunopurification of hepatitis B surface antigen
Tobacco	IgG (hepatitis B virus)	Therapeutic

Antibodies: a compelling success story

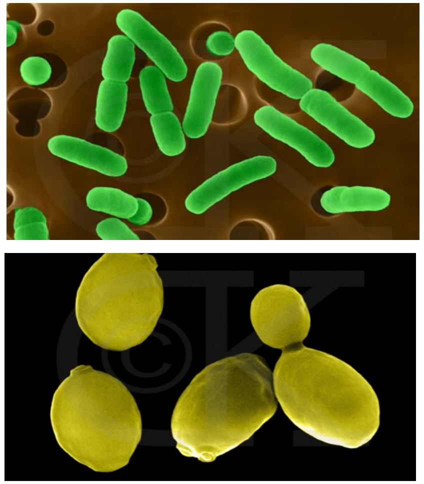
- high specificity: *in vitro* and *in vivo* diagnostics
- low toxicity: therapeutic applications
- high drug approval rates (24 approved mAbs)
- major products in biotechnology (~240 in clinical trials)
- inherently stable human proteins
- injectable, topical and oral applications
- applicable for chronic conditions
- potential long-lasting benefits



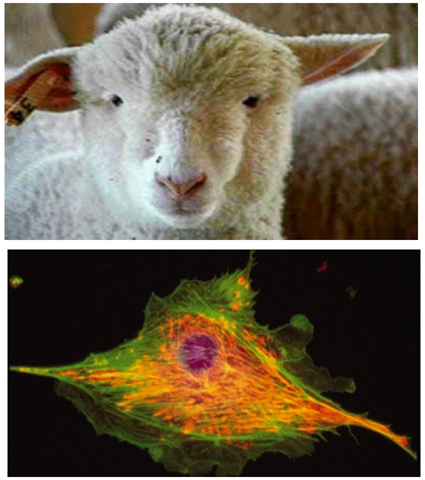
Production Costs for Antibodies

Production costs	cost in $ / gram
hybridomas	1000
transgenic animals	100
transgenic plants	10

Source: Daniell et al. (2001) TIPS 6, 219-226



E. coli & yeast



Tr. animals and animal cells

Transgenic plants

Edible Vaccines

One focus of current vaccine effort is on hepatitis B, a virus responsible for causing chromic liver disease. Transgenic tobacco and potatoes were engineered to express hepatitis B virus vaccine. During the past two years, vaccines against a *E.coli* toxin, the respiratory syncytial virus, measles virus, and the Norwalk virus have been successfully expressed in plants and delivered orally. These studies have supported the potential of edible vaccines as preventive agents of many diseases.

There is hope to produce edible vaccines in bananas, which are grown extensively throughout the developing world.

GENETIC ENGINEERING NEWS
Volume 19, Number 3
February 1, 1999

Cancer Vaccine Made in A Plant-Based System

Biosource and Stanford University Achieve a First in An Animal Model

A research team says it has produced a protein-based tumor-specific vaccine for the treatment of malignancies via a plant-based transient expression system.

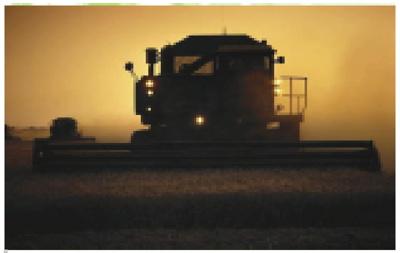
The collaborative group, led by Daniel Tusé, Ph.D., at **Biosource Technologies, Inc.** (Vacaville, CA), and Ronald Levy, M.D., Ph.D., at Stanford University (Stanford, CA), describe a strategy for individualized treatment of indolent non-Hodgkin's lymphoma (NHL) in a mouse model system. Though

Biosource researchers used tobacco plants as an alternative mechanism for antibody production. The researchers removed malignant B cells from laboratory mice and then isolated the gene for a small piece of the surface markers that are specific to these cells. They inserted this gene into tobacco mosaic virus and then infected tobacco plants.

Plants are also being genetically engineered for:

- **Biofuel production (e.g., lower lignin, lower recalcitrance)**
- **Phytoremediation (i.e., bioremediation using plants)**
- **Biopolymers (i.e., biodegradable plastics)**

Roadmap Plants for the Future



Efficient agriculture

- Bt technology
- Herbicide resistance

Health food and quality

- Amino acids
- Oil
- Starch

Plant protection

- Viruses
- Nematodes
- Fungi
- Insects

Plant production platforms

- Vitamines
- Fatty acids
- Enzymes
- Bio-polymers
- Pigments
- Pharmaceutical products
- Fibers

Stress resistance

- Cold
- Drought
- Salinization

1997 — 2005 — 2015 — 2025

Plants for the future