BIOTECHNOLOGY – STATE AND PERSPECTIVE

created by Morenko A.

Full PhD

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The Lecture Will Cover the Following Topics:

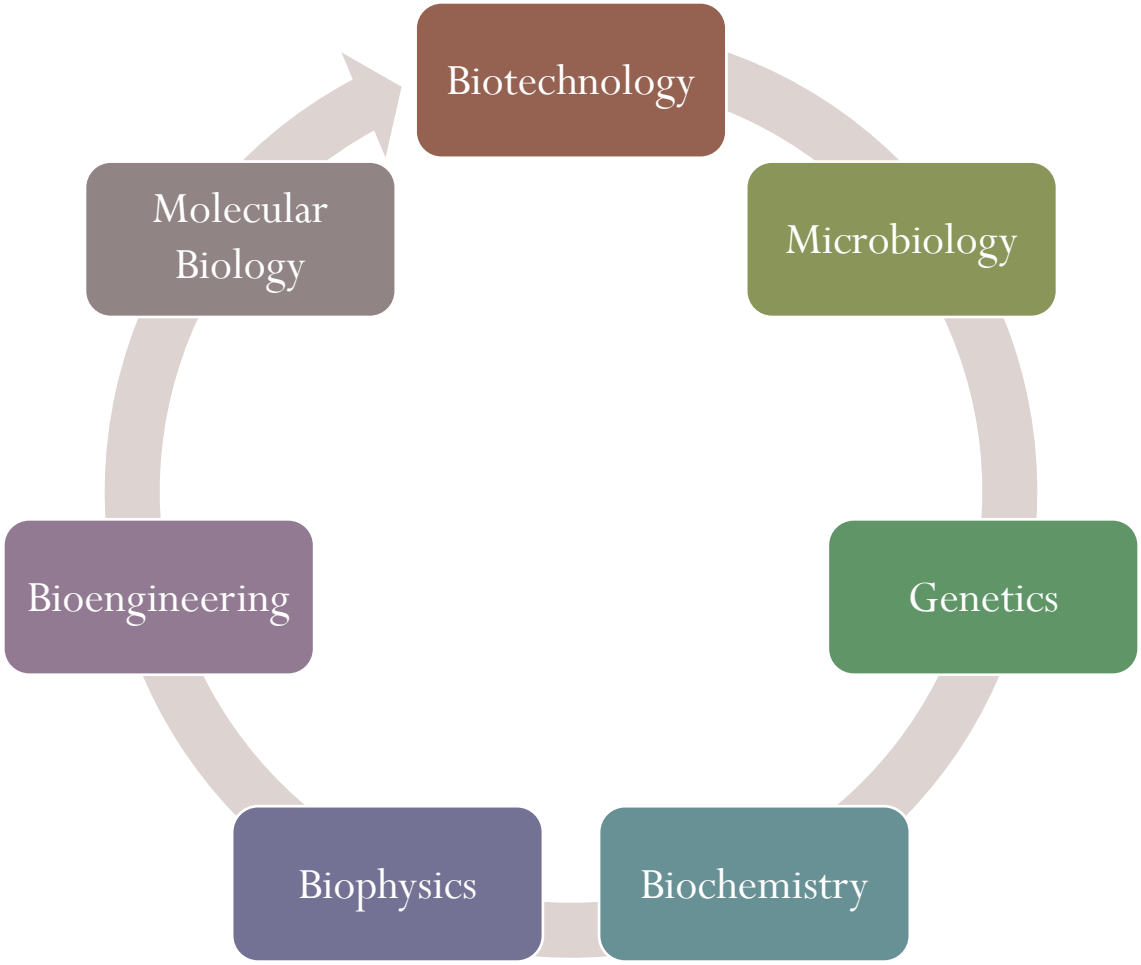
- products and services to the community.
- not all biotech is new (fear of new technology).
- advantage and potential disadvantages (based on today's perspective - theoretical risks).
- future trends in biotechnology.
- ethical, social, and economical implications by applying biotechnology in society.
- new production methods in genomic science and its uses.

What is Biotechnology ?

It uses living organisms to carry out defined biochemical processes for industrial applications.

Living organisms can be used in the manufacturing or service industry (like genetic fingerprinting, gene therapy, etc.).

Which Branches are Involved in Biotechnology ?



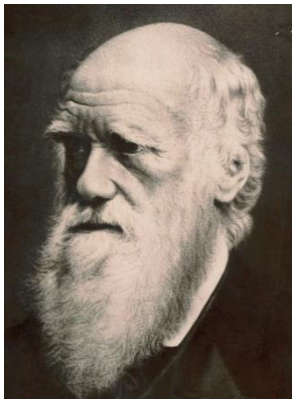
Some Ancient Biotechnologies:

- **beer brewing** was already known to the Babylonian's (6000 BC); even in mediaeval times;
- **alcohol fermentation** was practiced by the catholic monks, or in district distillations of ancient China;
- **Breadmaking skills**: the inoculation of fresh dough with one of the previous day was known to the ancient Europe, and even to Egyptians, some 4000 years ago (germination of barley);
- **Diary products**: the production of yogurt, cheese, kefir, and even vinegar was known for centuries; the monks were even familiar with the beneficial effects of these milk products.

16 – 18 CE: beginning of the Industrial Revolution in Europe

Anton Van Leeuwenhoek, **in 1663** was the first person who observe living cells, a microscope builder. **In 1674** he discovered bacteria from a sample of saliva from his mouth .

In 1797, **Edward Jenner** inoculates child to protect him from smallpox



In 1859 Charles Darwin published the theory of evolution through natural selection

In 1857 Louis Pasteur proposes microbe theory for fermentation.

In 1865 Gregor Mendel published the results of his studies on heredity in peas

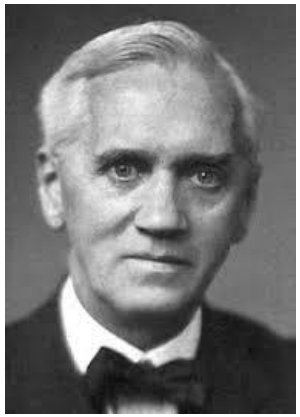
Further Milestones in Biotechnology:

Hance Buchner

Eduard Buchner

1897: Buchner Brothers - laid the foundation for enzyme technology. They isolated enzymes from yeast in a clear and scientific manner;

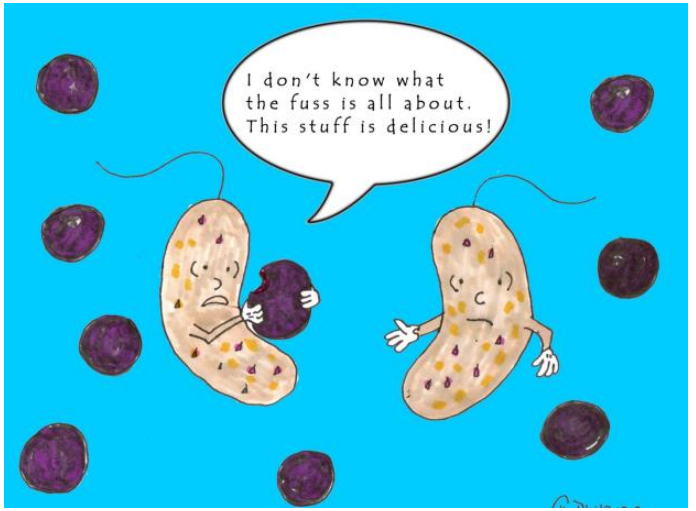
1929: Discovery of Antibiotics by **Alexander Fleming** - many lives were saved during WWII because antibiotics were produced on a large scale.



- **1950** and onwards - technical advances like the discovery and use of the SEM (Scanning EM) and the TEM (Transmission EM); biological discoveries and DNA research; in 20 years, around 20 Nobel prices were related to that scientific branch;
- **1970's -1980's** - industrial applications were launched as the industry recognized the potentials and economical benefits and profits of biotechnology.

Fields of Interest

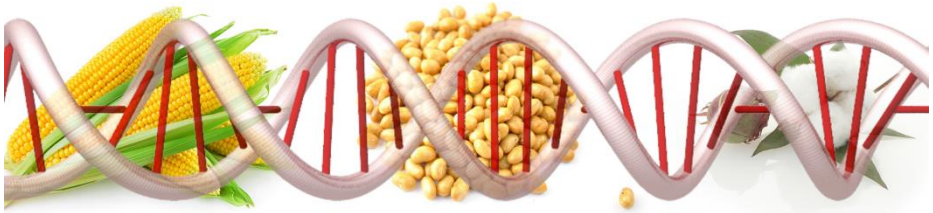
- Biotechnology aims at a cleaner way to produce food, generate medicines that can treat new and old diseases;
- Secondary environmental effects (as observed with oil spills) in which microbes are used in the cleanup process;



All these beneficial effects seem to ignore the reverse side effects.

Skeptics

- *Agriculture*: Development of GM-foods and other alternative products raised concerns among the general public; secondary environmental effects due to the introduction of raw GM-products into the wild are poorly understood;



- *Industry in general*: new, highly skilled jobs will be created, cheaper ways in manufacturing certain goods, however current concerns about the clean-up of wastes could be talked with biotechnology.

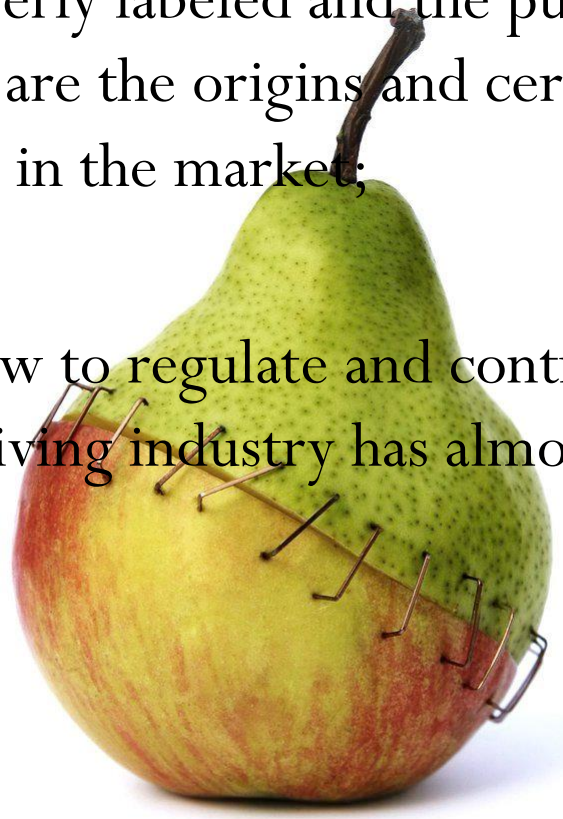
Skeptics

General public:

- new products are viewed at with suspicion:
- GM-foods are not always properly labeled and the public often doesn't even know what are the origins and certain aspects of a particular product in the market;

Legislative base:

laws regarding GM-products, how to regulate and control the new products are not existing, giving industry has almost an open door to operate.



Why Biotechnology is Very Attractive to Industry ?

- It can utilize *waste products* as a raw materials to create new products; these raw substances are cheaper to produce than most traditional industrial products.
- It requires very low energy requirements - most industrial applications operate at low temperatures;
- it does not need large power plants which makes it applicable for less developed countries;
- it can be used not only for the benefit of mankind but also as a tool in the "bio-warfare programs", for example.

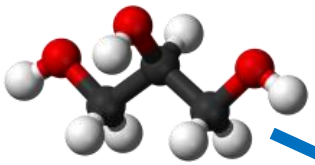
During WWII

The yeast of soy beans

Exposing to hydrogen sulfide

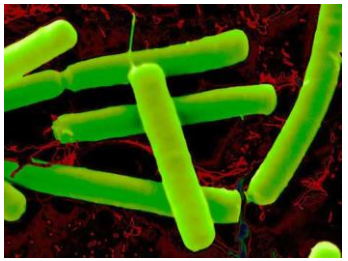
Vegetable oils

Vegetable Glycerol



Production of explosives

During WWII



Fermenting capacities of Clostridium sporogenes bacteria

An undesired byproduct of this fermentation

H-GAS

Producing ammunition

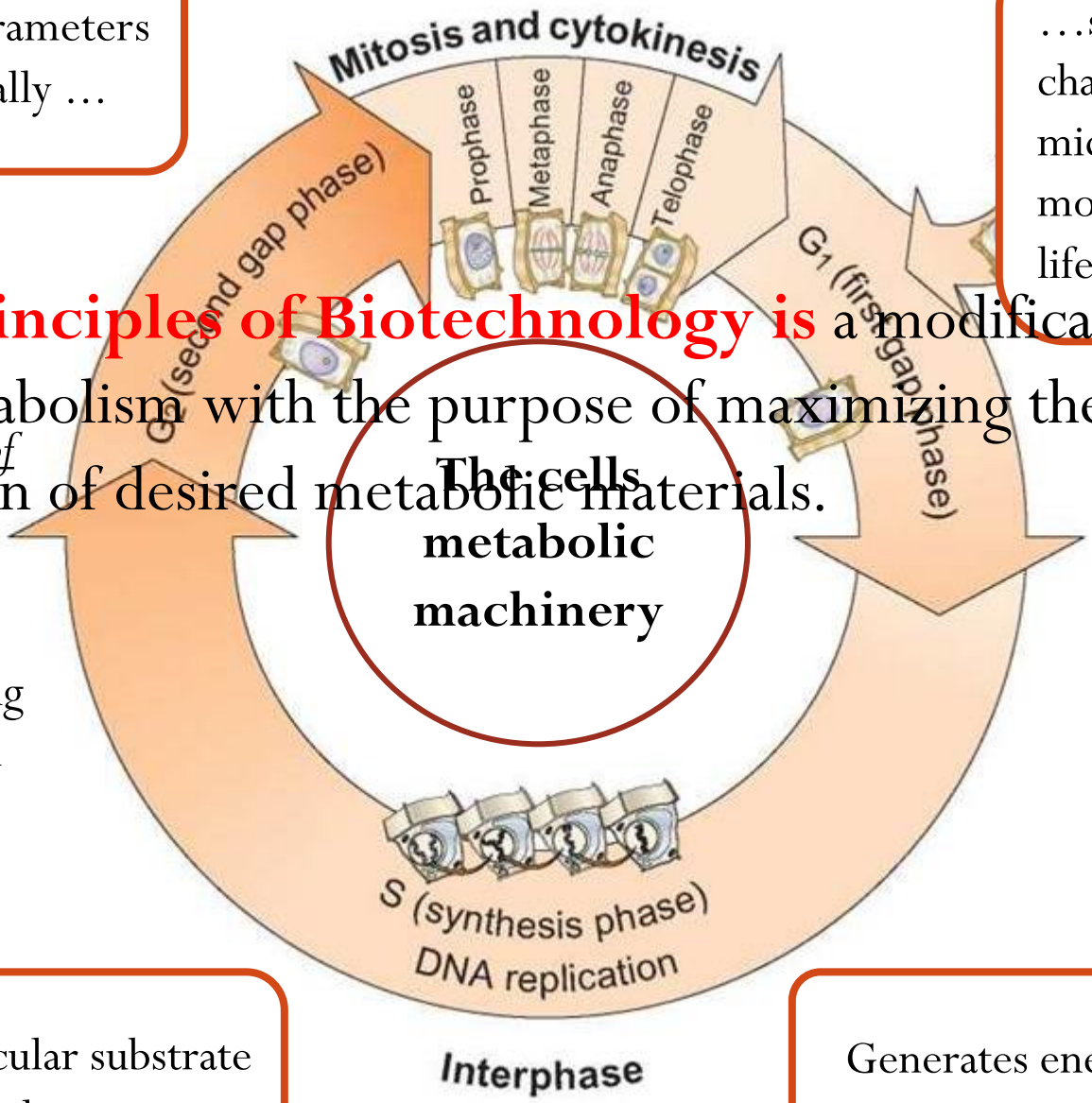
H-gas was the cause for violent explosions that destroyed the entire production facilities.

When abiotic parameters change drastically …

…some protective changes in the microorganism modifies its lifecycle,

Basic Principles of Biotechnology is a modification the cell's metabolism with the purpose of maximizing the production of desired metabolic materials.

Bio-engineers *explore the biochemical steps of cells* in order to make particular products (by carefully selecting distinct cell-own enzymes)

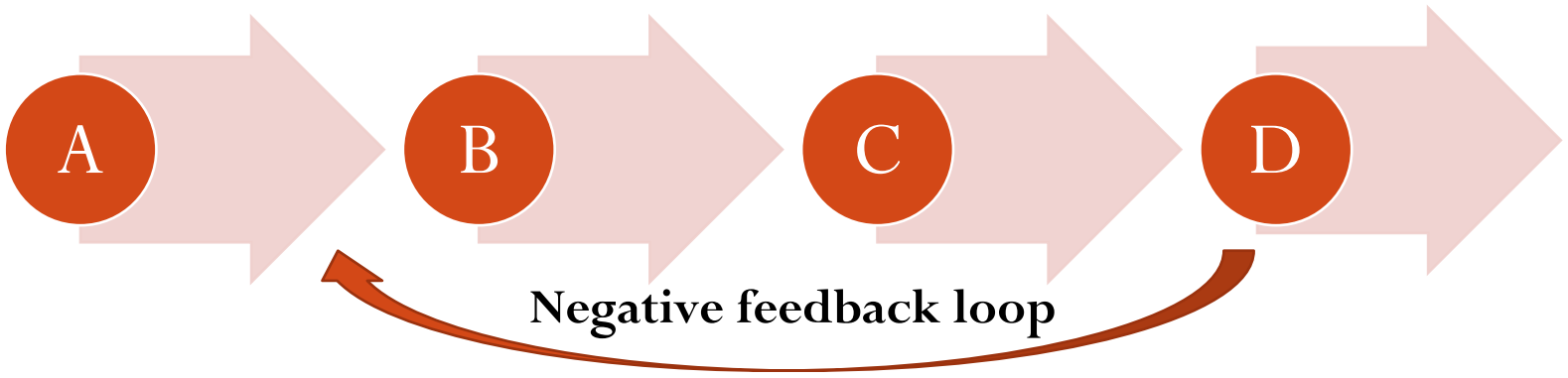


Spore formation in bacteria, accelerated aging, build-up of antibiotics, etc. can be changed

Feeds on a particular substrate and degrades it.

Generates energy, biomass.

Intermediates of a cell's metabolic pathway

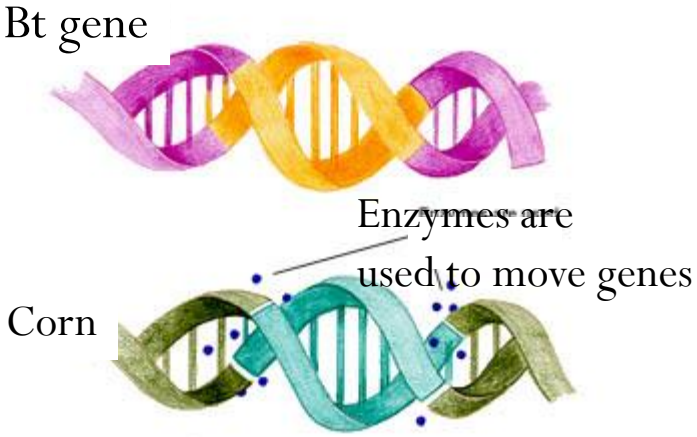


Biochemistry uses these intermediates to influence the production line by regulating and controlling the enzymes, i.e. the cell metabolisms by the way of the ***negative feedback loop***.

This principle is based on application of **Genetic Engineering**

The cells are modified in a way to lower the losses of intermediate reactions in order to maximize the desired end product "D".

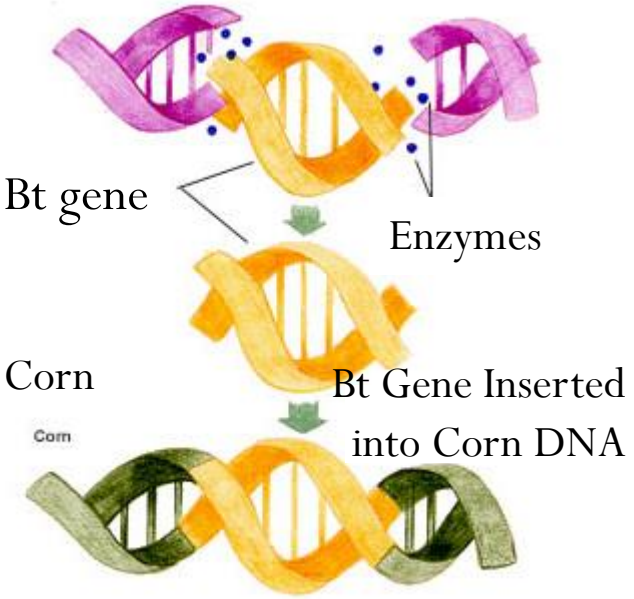
Being self-regulated, the negative feedback loops have the advantage of avoiding waste of energy and the sudden stop of production.



To obtain a distinct product that is not generated by a particular organism it requires some changes and modification of the cell's DNA in order to select the one with the best qualities.

GM Screening methods

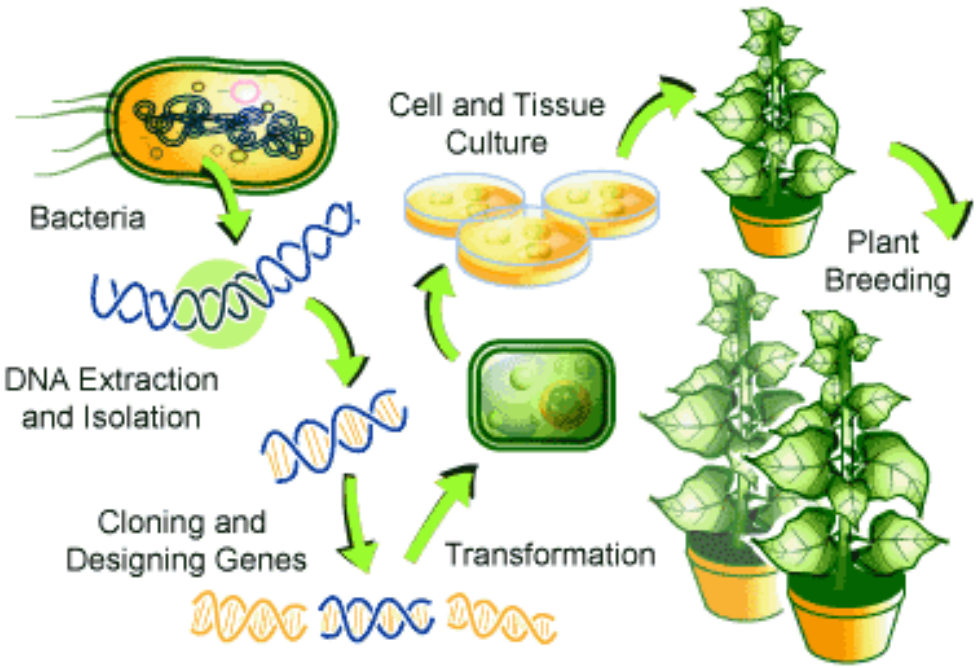
Genetic engineering is far more reliable than exposing the microorganisms to energy-rich electromagnetic radiation (X- or g-rays);
So, mutations are not brought about by random impacts, but rather directed by introducing a selected gene-sequence into the cells genome.



A forcefully induced mutation.
A distinct gene-sequence is introduced into a third cell to trigger changes until the desired cell type is obtained.

Gene & Protein

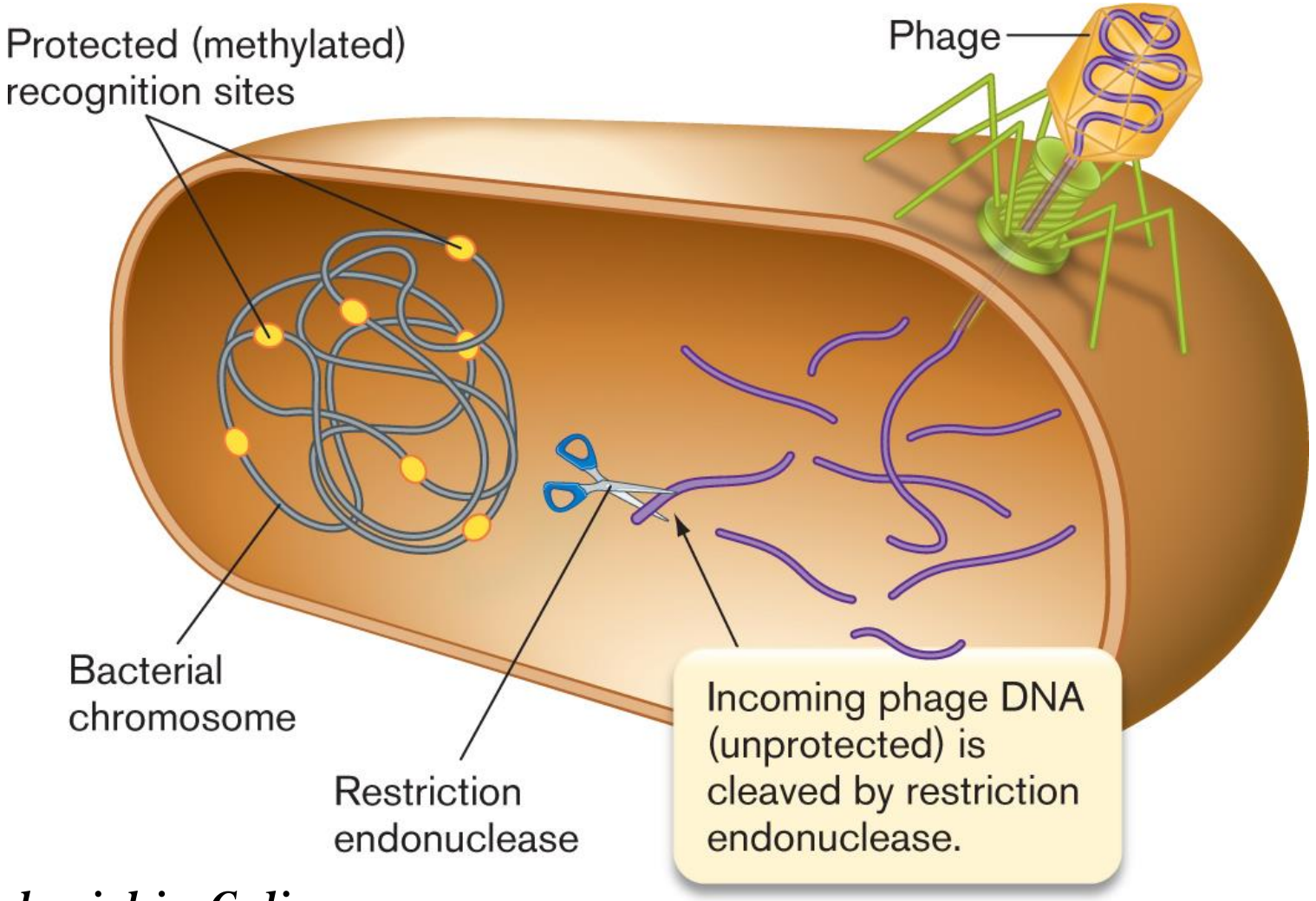
- Single gene sequences within the genome work together to produce a functioning protein.
- **The tasks of bio-engineers** are to localize the genes that code for new enzymes and introduce them into a suitable host.



The introduction of the new genes force the microorganism to generate substitute products.

That organism get a totally new set of characteristics and properties..

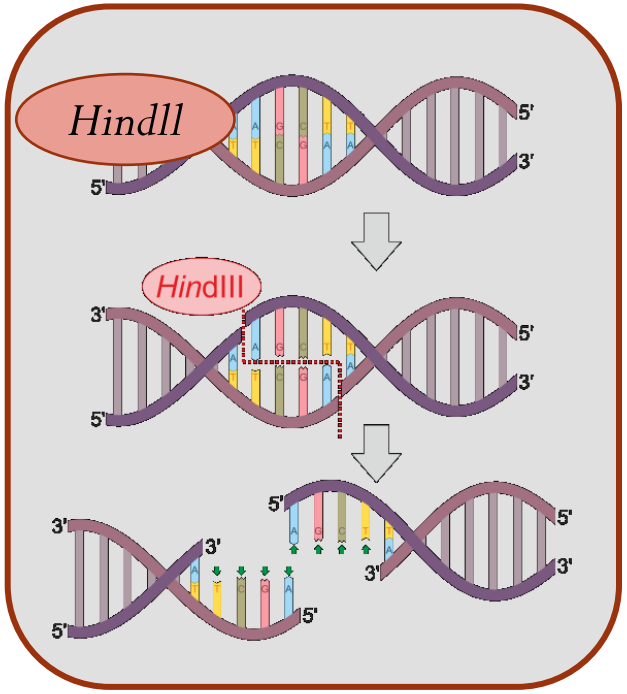
How Its Done?



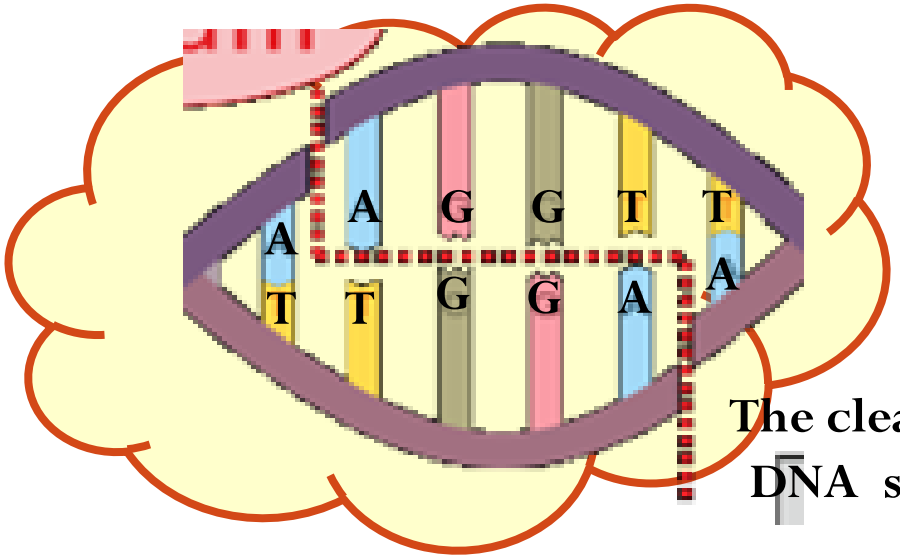
Escherichia Coli

How Its Done?

Virus



Consequently the viral DNA is not able to launch the reverse transcriptase in order to use the bacteria's own enzymatic machinery to produce more viruses.

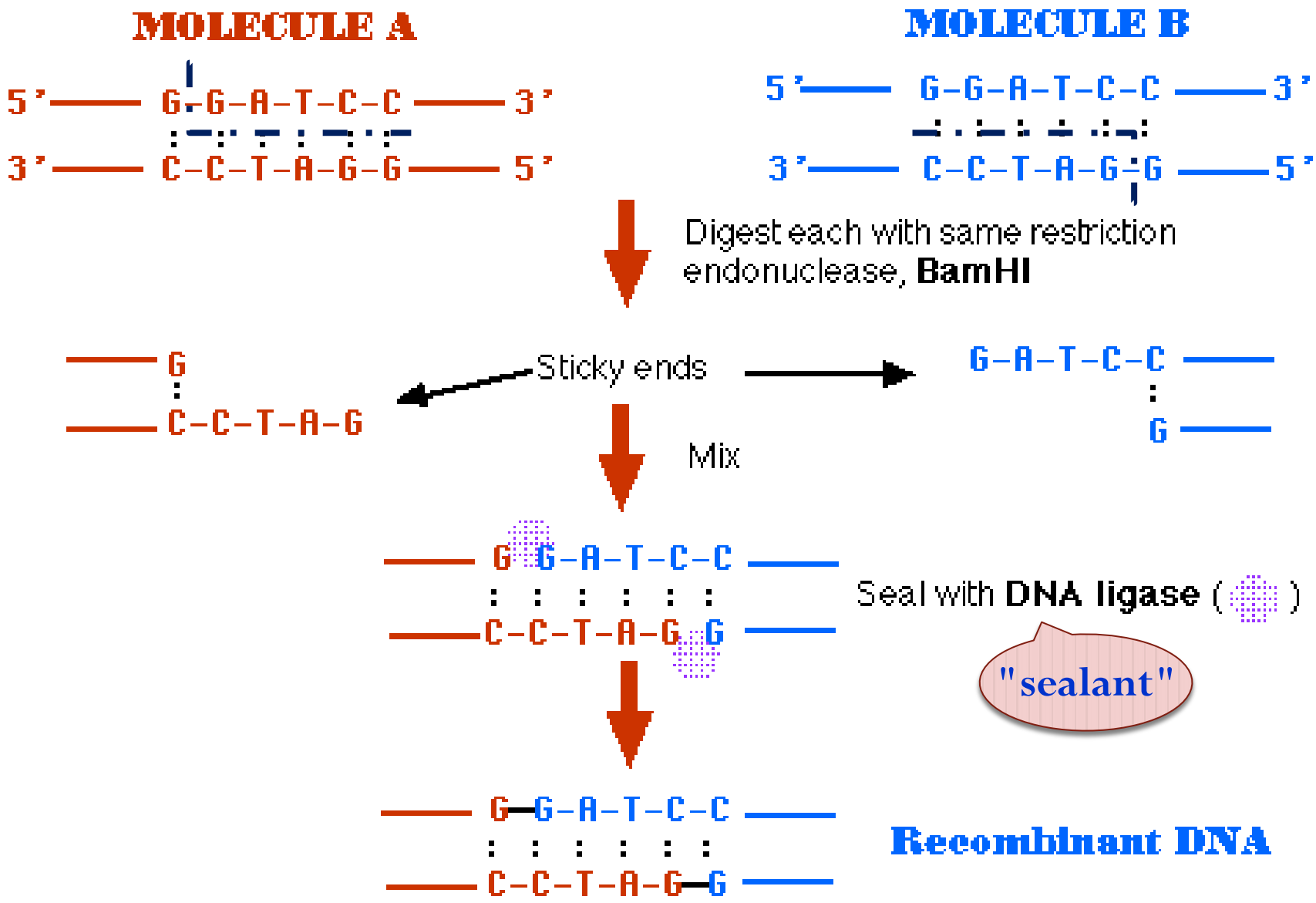


The cleavaged DNA strands

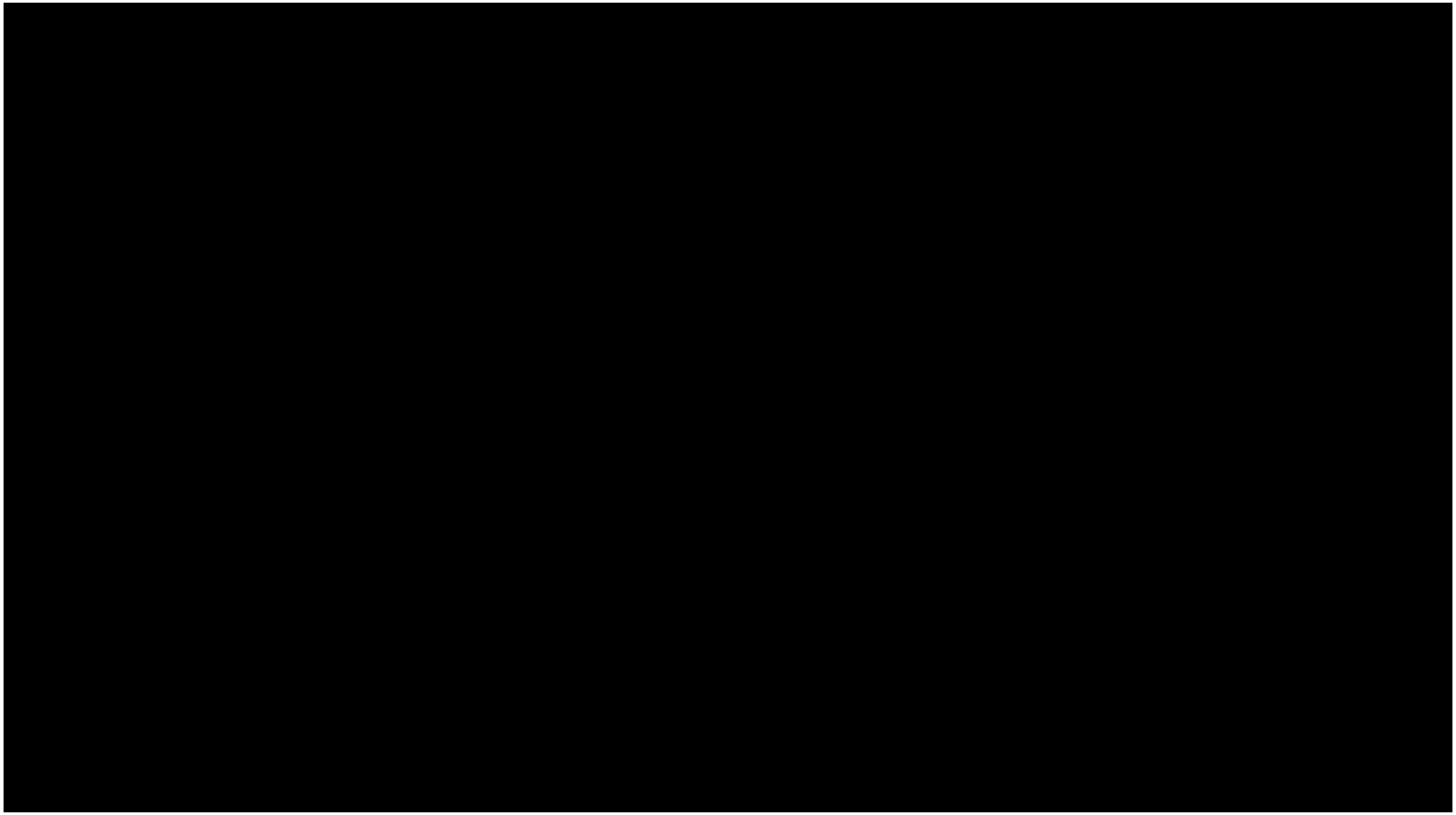
Restriction Enzymes, were discovered in the early 1970's.

So far, many different restriction enzymes have been identified

Making Recombinant DNA



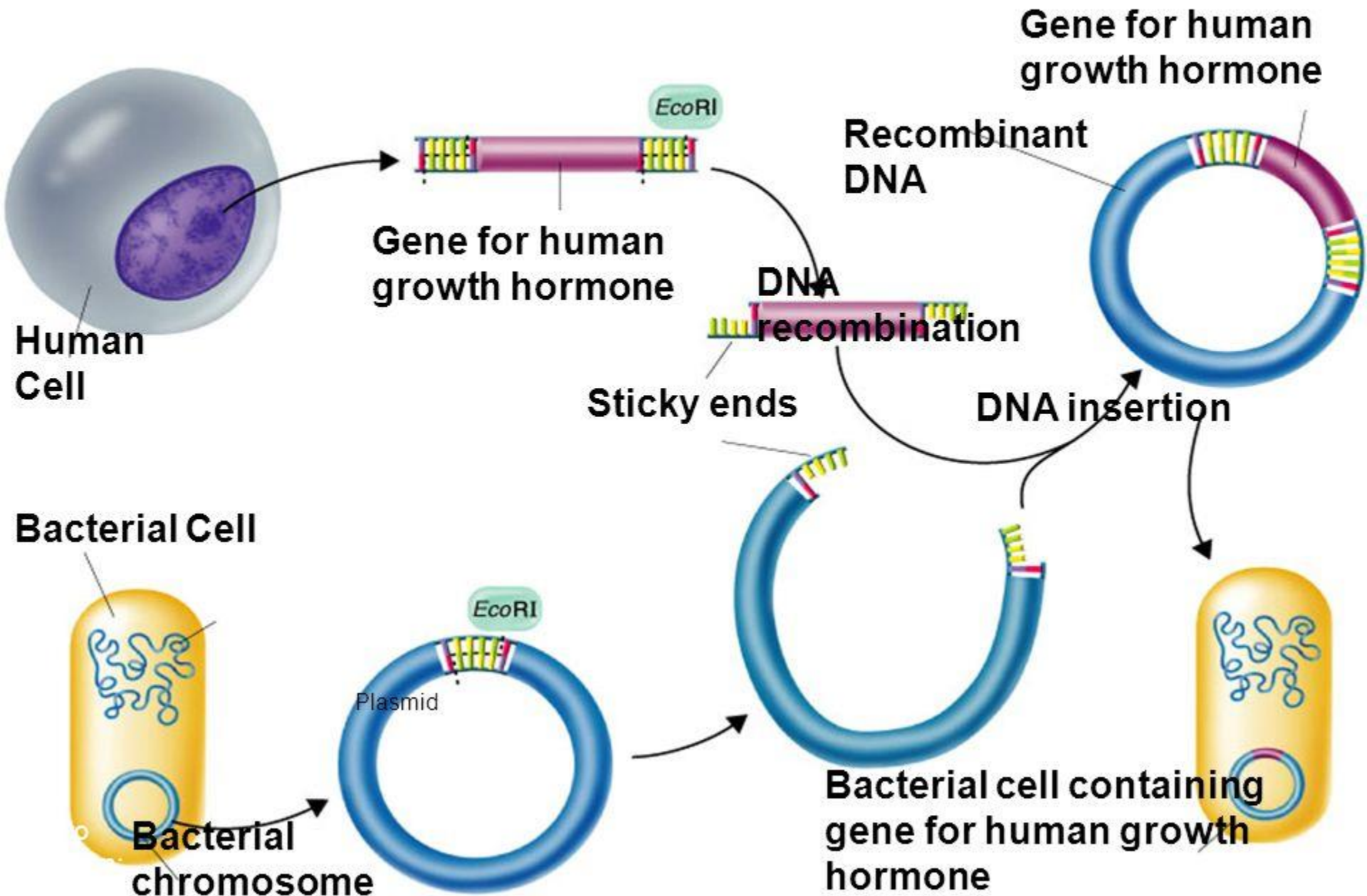
PCR and Recombinant DNA



Making Recombinant DNA

Transformation of E.Coli with Plasmid DNA

Making Recombinant DNA

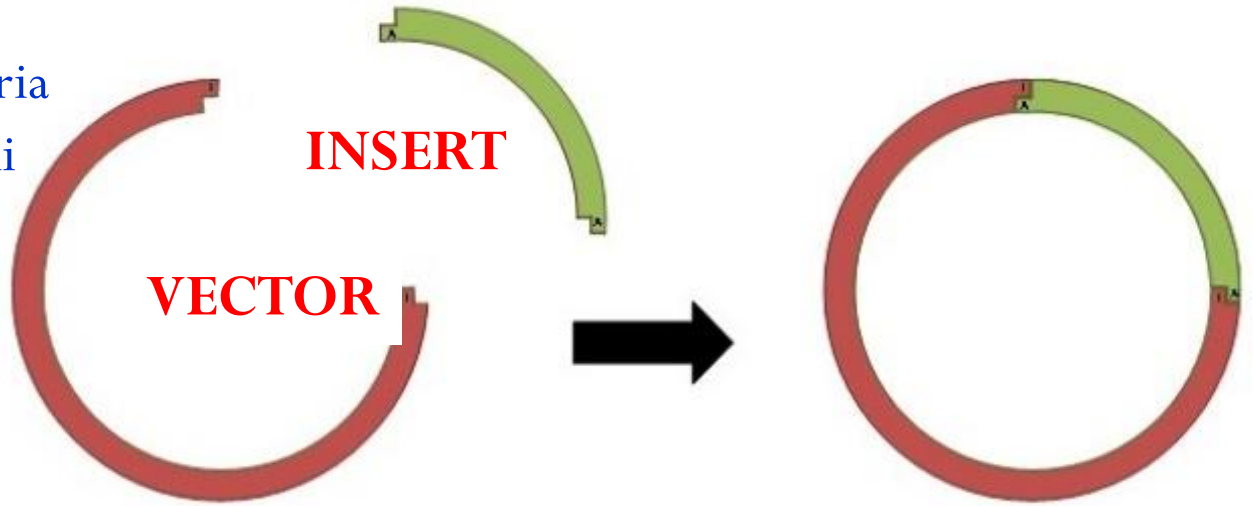


Recombinant DNA

- The basic tool to modify an organisms genome is achieved with **recombinant DNA**.
- Recombinant DNA is made by splicing a foreign DNA fragment (**insert**) into a small replicating molecule (such as a plasmid, **Vector**), which will then amplify the fragment along with itself resulting in a molecular "clones" of the inserted DNA fragment.

Amplify is an increase in the frequency of a gene, as a result of replicating a DNA segment.

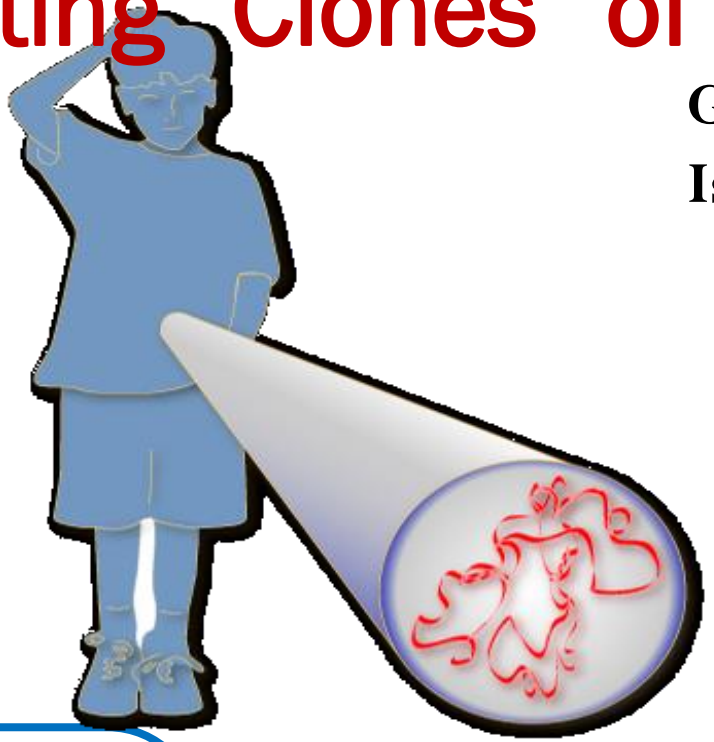
Bacteria
E.Coli



E.coli is the most commonly used bacteria in gene-engineering. It yields immediate results as it multiply quickly.

Generating "Clones" of the Gene

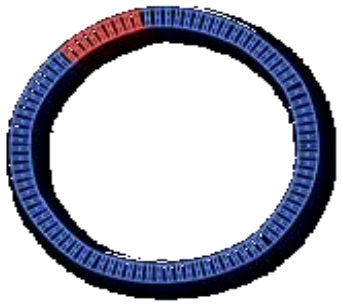
A clone is a group of cells, organisms, genes, etc. that are an exact copy of each other.

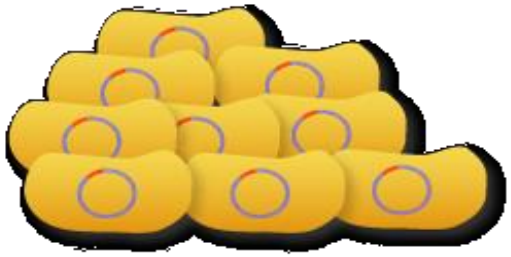


Human Genome

Gene of Interest
Isolated from Genome

Gene
Inserted
into Plasmid



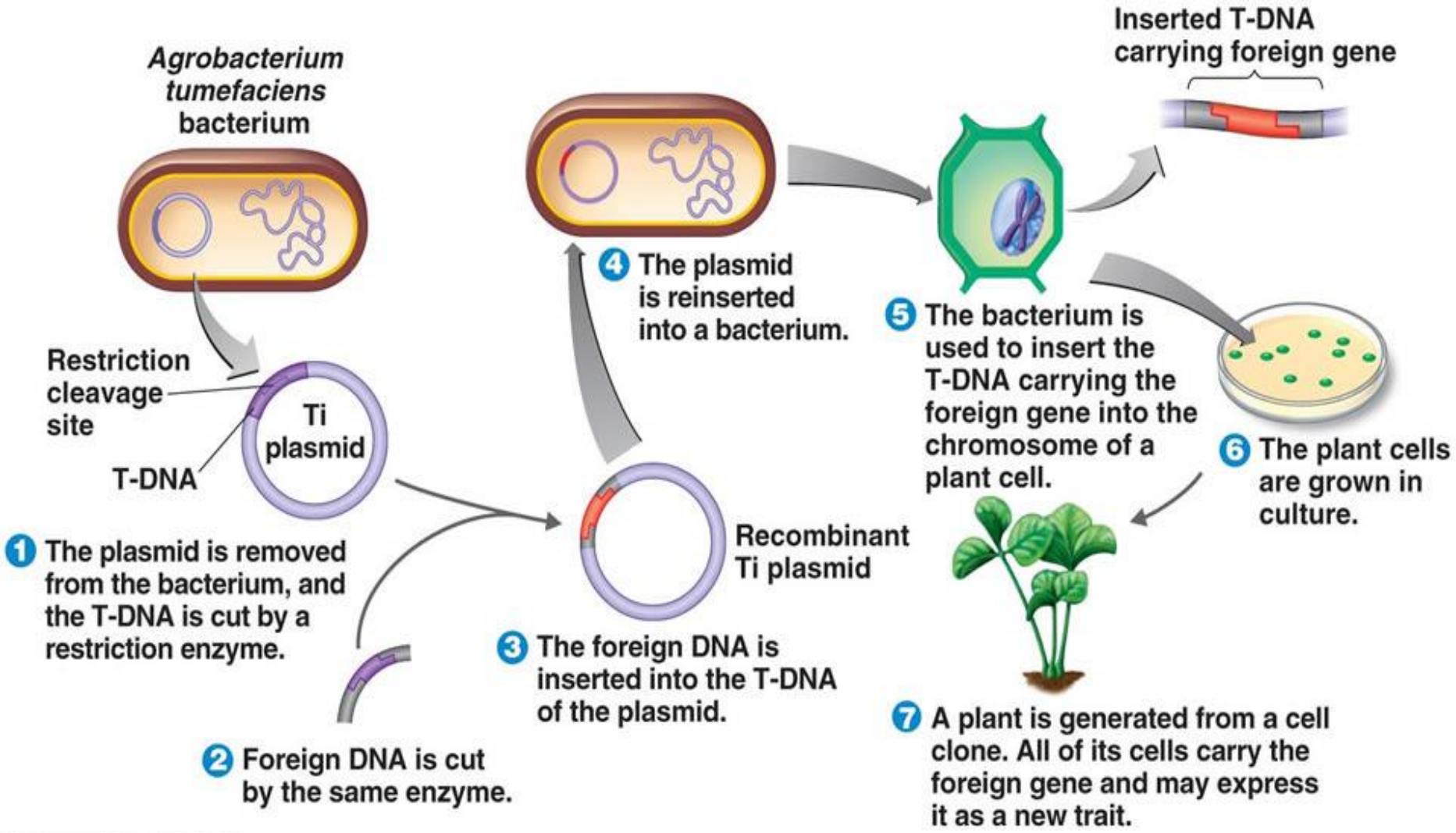


Plasmid Inserted into Bacteria Population
Generating "Clones" of the Gene

Cloning allows the amplification and recovery of a specific DNA segment from large, complex DNA, sample such as a Human genome.

How Does it Work in Plant Selection?

Cells from plants with desirable characteristics can be cloned to produce many identical cells. Plant cells can be engineered by using the Ti plasmid vector. The Ti plasmid is carried by *Agrobacterium tumefaciens* and causes *Crown gall* disease.


Agrobacterium tumefaciens bacterium
Restriction cleavage site
T-DNA
Ti plasmid
1 The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2 Foreign DNA is cut by the same enzyme.
3 The foreign DNA is inserted into the T-DNA of the plasmid.
Recombinant Ti plasmid
4 The plasmid is reinserted into a bacterium.
Inserted T-DNA carrying foreign gene
5 The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.
6 The plant cells are grown in culture.
7 A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.

How Biotech Has Transformed the Industry

Advantages

By adding faster synthesizing machines into a slow working host cell, the metabolic processes and the activation of key enzymes are boosted significantly. Once the proper cell density is reached, the production can get started.

Disadvantage

Microbial activity causes the production of substantial amounts of heat; it is necessary to provide cooling equipment in order to avoid overheating, which would ultimately terminate the bioreaction

Cooling systems of fermentation tanks are very expensive. However, substituting genetically modified thermophobic microorganisms with thermophilic ones can help to reduce costs in cooling equipment.

Social Implications – Who is Afraid of Biotech?

- A biodiversity treaty was drafted in Rio de Janeiro in late 1992; it complains the principles of protection of flora and fauna; a major goal is a stop to the ongoing trend of extinction.
- So far the *US Natural Academy of Sciences* concluded that selective breeding did not harm diversity in crops, plants, grass, etc.; extrapolation of this conclusion to the GM sector is not possible since the term breeding is expanded to interspecies crossing!
- In 1998 a team of scientists of about 100 different organizations met to discuss GM-products. They stated that ratification of the treaty by UN is possible, but it cant be excluded that biotech will not have a harmful effect upon natural plants and animals (potential effects of GM-products are not known).

Laws and Patents – "Can We Pay for Life"?

A patent represents the exclusive right to manufacture or use a product.

Many companies refrain from applying for a patent and try to keep their product contents secret.

In 1988, the USA patented its 1st ***transgenic mouse*** - used for standardized cancer research.

In Germany, the transgenic bull ***Herman*** was born. ***Herman*** could not be patented in EU.

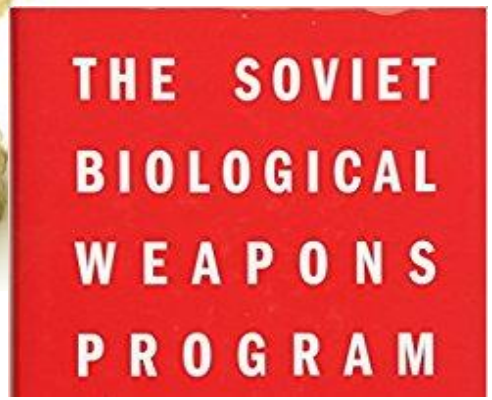
Patenting laws in EU lack behind those of the USA. Problem: who gets the royalties for every calf that originates from *Herman* and what will happen when the bull (or at least his sperm) eventually goes abroad?

Ethical Issues

- Is it morally justified to modify an animal for the sole goal to benefit humans? The transgenic mouse was created to suffer in order to obtain a standardize test animal for cancer research.
- Is it also morally justified to change the genetic diversity of our ecosystem?
- Should the consumer know what a GM-product is made of (labeling of all GM-products)?

GM Organisms Released into the Environment

- The safety code is similar to those used in the nuclear industry: the use of high tech is necessary to prevent accidents - even though there is a very low rate of risk.
- Killing GM organism at the end of the production cycle.



Was during the cold war and lasted till 1993

Substances along with concentrated acids and other toxic chemicals

Drilling holes at an island in the Aral sea

In 1995, The Kazakhstan government asked the US department of Health to check those sites. To the surprise, some cultures survived the chemical attack and were still found to be functional (Biohazards, by author Alibek Ulak).

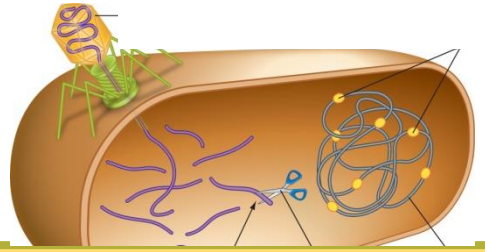
GM Organisms Released into the Environment



E.coli **and yeast** are the most commonly used microorganisms in biotechnology

Once this modified bacteria is released into the environment, scientists do not know if wild strains of this microorganisms will get this gene via bacterially mediated plasmid transfer.

If these strains are seepaged accidently, there will be an uncontrolled release into the environment



A way to control it is to incorporate suicide genes into the bacteria in order to trim down survival of the strain outside the controlled containment

Competition with Natural Species:

Better fitted GM microorganisms will probably outcompete the wild strains. How can such an event be monitored?

GM products:

have to be different from the natural analogue,

produce higher yields, larger product-biomass, etc.;

have highly resistance to several ambient factors,

are a lot easier to handle, as their reproductive DNA is passed on only via a suitable fe/male individual.

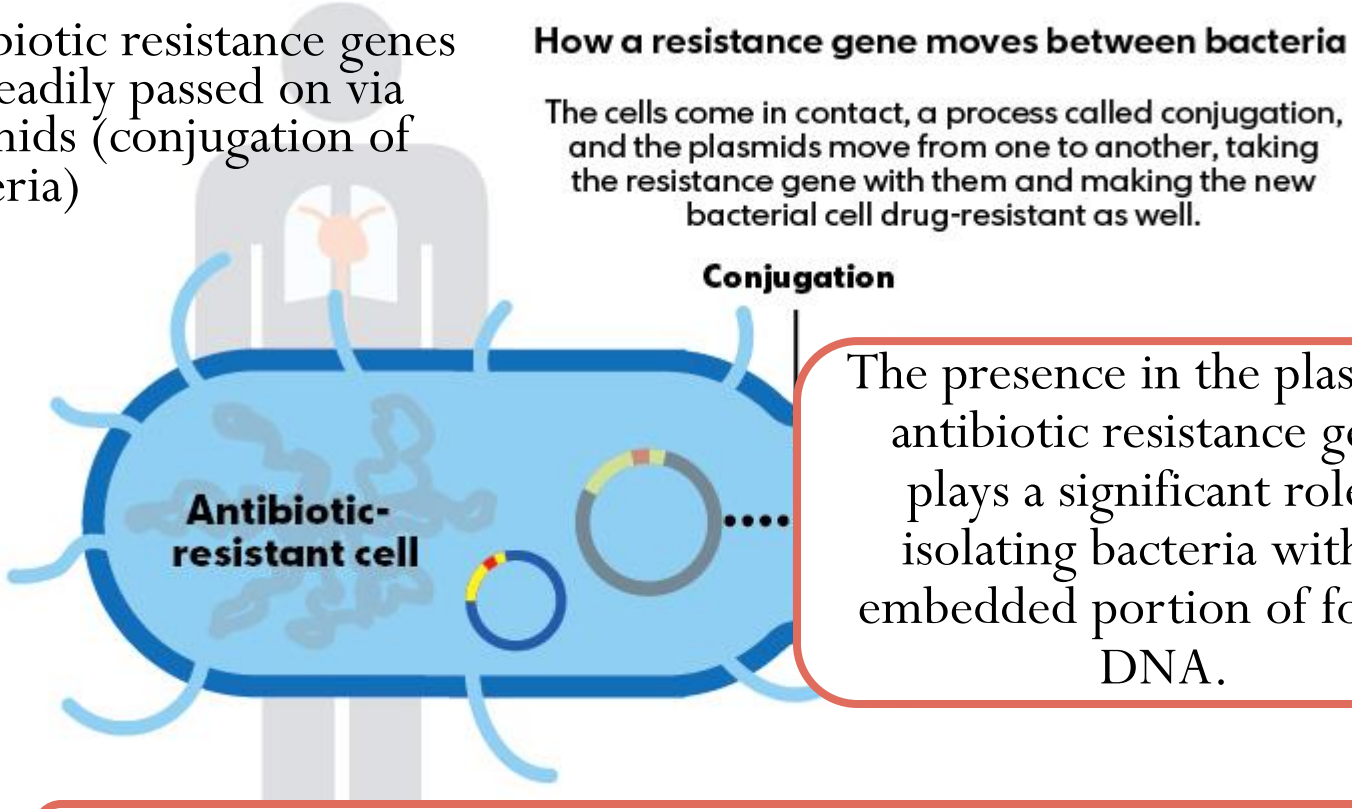
Seaweed *Calerpa taxifolia,* biotechnically modified

It has been accidentally spilled into the Mediterranean sea

So far no remedy has been found to eradicate this mutant.

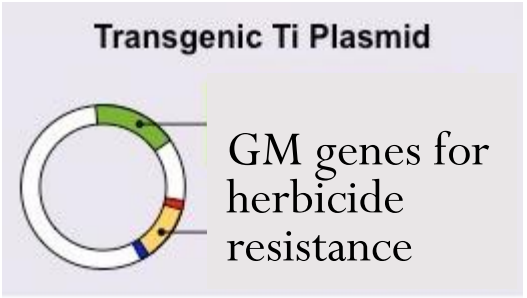
Competition with natural species can be realized using cloning vectors. In such a way disease transmittance may be facilitated in microorganisms.

Antibiotic resistance genes are readily passed on via plasmids (conjugation of bacteria)



The presence in the plasmid of antibiotic resistance genes plays a significant role in isolating bacteria with an embedded portion of foreign DNA.

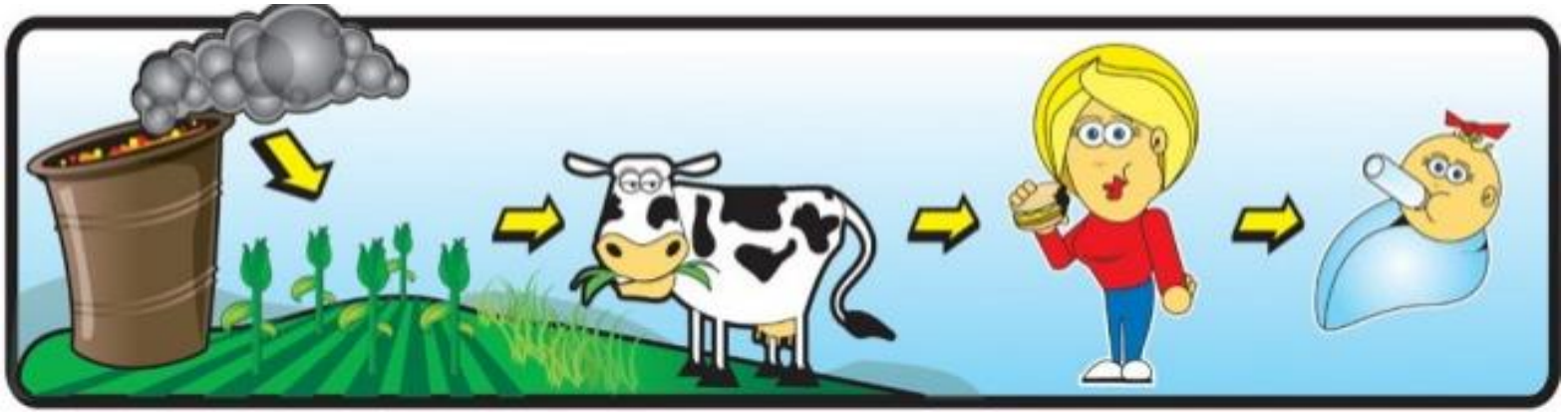
Likewise, usually non-toxic strains may catch up the toxic properties by the same way.



Production yield Resistance to weed and predators

Bioaccumulation seem to be a serious problem.

- Scientists suggest that initial trials to generate such plants should be stopped, as acting metabolites accumulate along the food chain.



Turn back the clock 9000 years

NATURAL "CORN", 7000 B.C.

Peel it by hammering repeatedly with a hard object

Tasted like very dry, row potato

19 mm

5-10 very hard kernels

8 known varieties

Only found in Central America

75% water

1,9% sugar

23% other mostly starch

ARTIFICIAL CORN, 2014

Steam cook in minutas

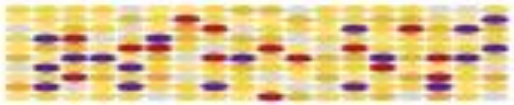
190 mm

Much more sweeter

Avaiable in 5 colours

Easyer to peel

1000 times larger



More then 200 varieties

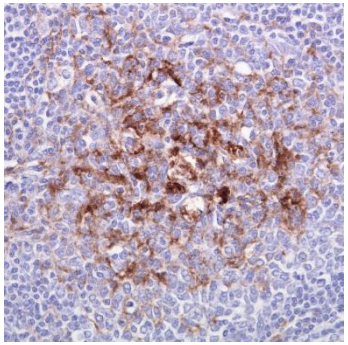
Grown in 69 counties

73,2% water

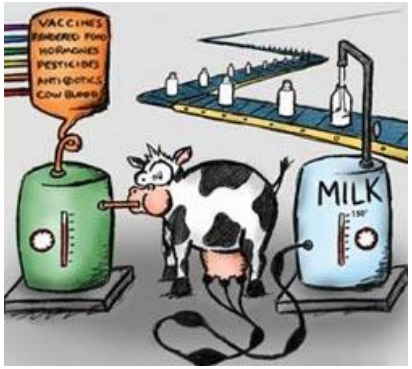
6,6% sugar

20,2% other still rich in starch

New Drugs in competition with natural species: new vaccines and drugs will be developed in the years to come; probably, these remedies will be "*a lot safer*" than conventionally produced ones; e.g.

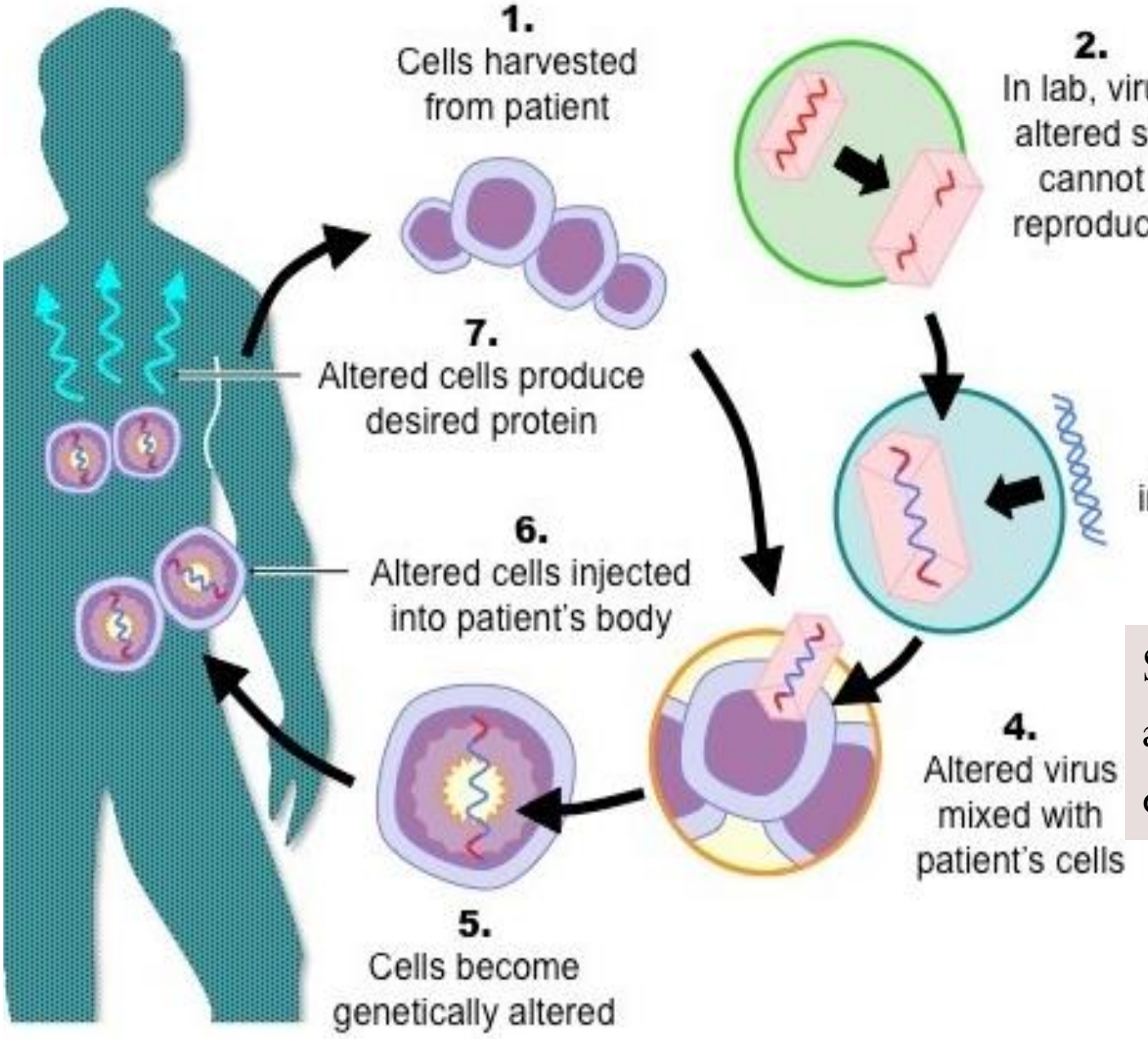


Tonsil Biopsy
in Variant CJD



- **Creutzfeld-Jacob-Disease (CJD)** is "definitely" transmitted by prion of BSE (bovine spongiform encephalitis) that occurs in natural growth hormones. **But!** synthetically generated hormones would have made transmission impossible.
- b) **BST (Bovine Somato Tropin)** is a hormone that causes cows to give 25% more milk and is widely used in USA-agriculture. **Unfortunately**, the use of it has severe side effects.

Somatic Gene Therapy - in short: extract a tissue sample, treat it and place it back to the organism

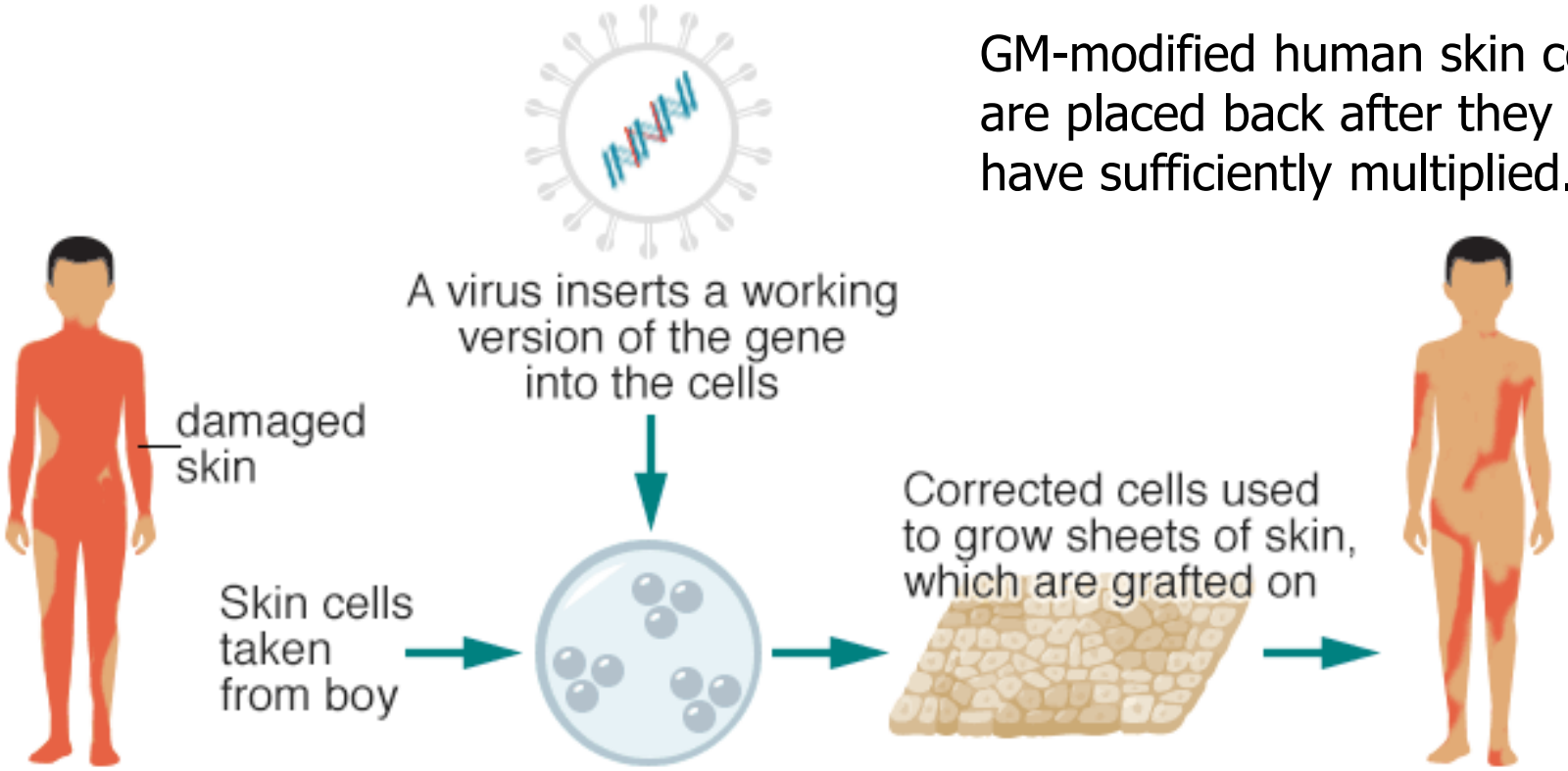


Potential applications of this technique involve cancer therapy.

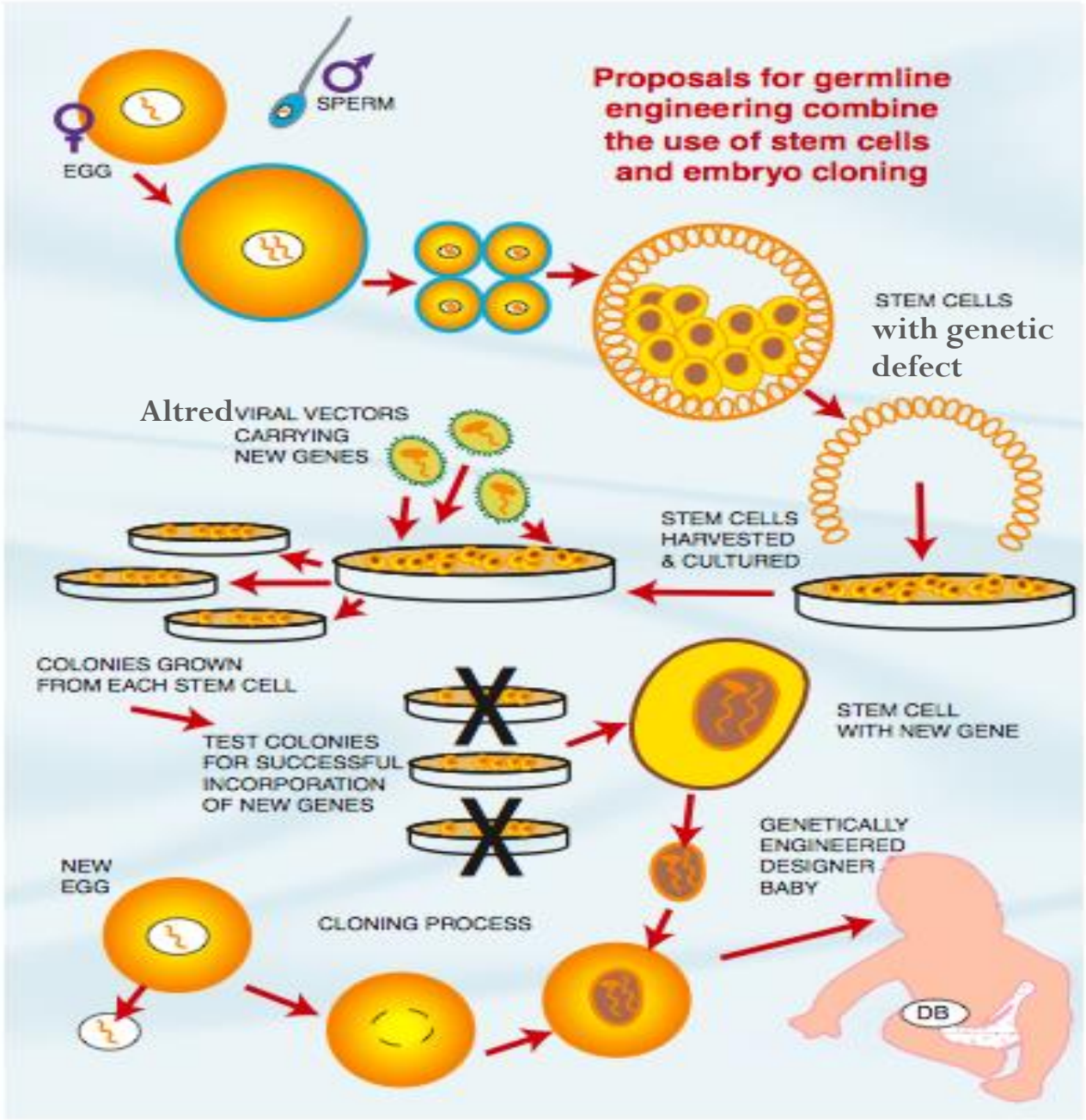
Since somatic cells are not handed onto offspring.

SKIN GRAFT – a somatic gene therapy to make new skin

GM-modified human skin cells are placed back after they have sufficiently multiplied.

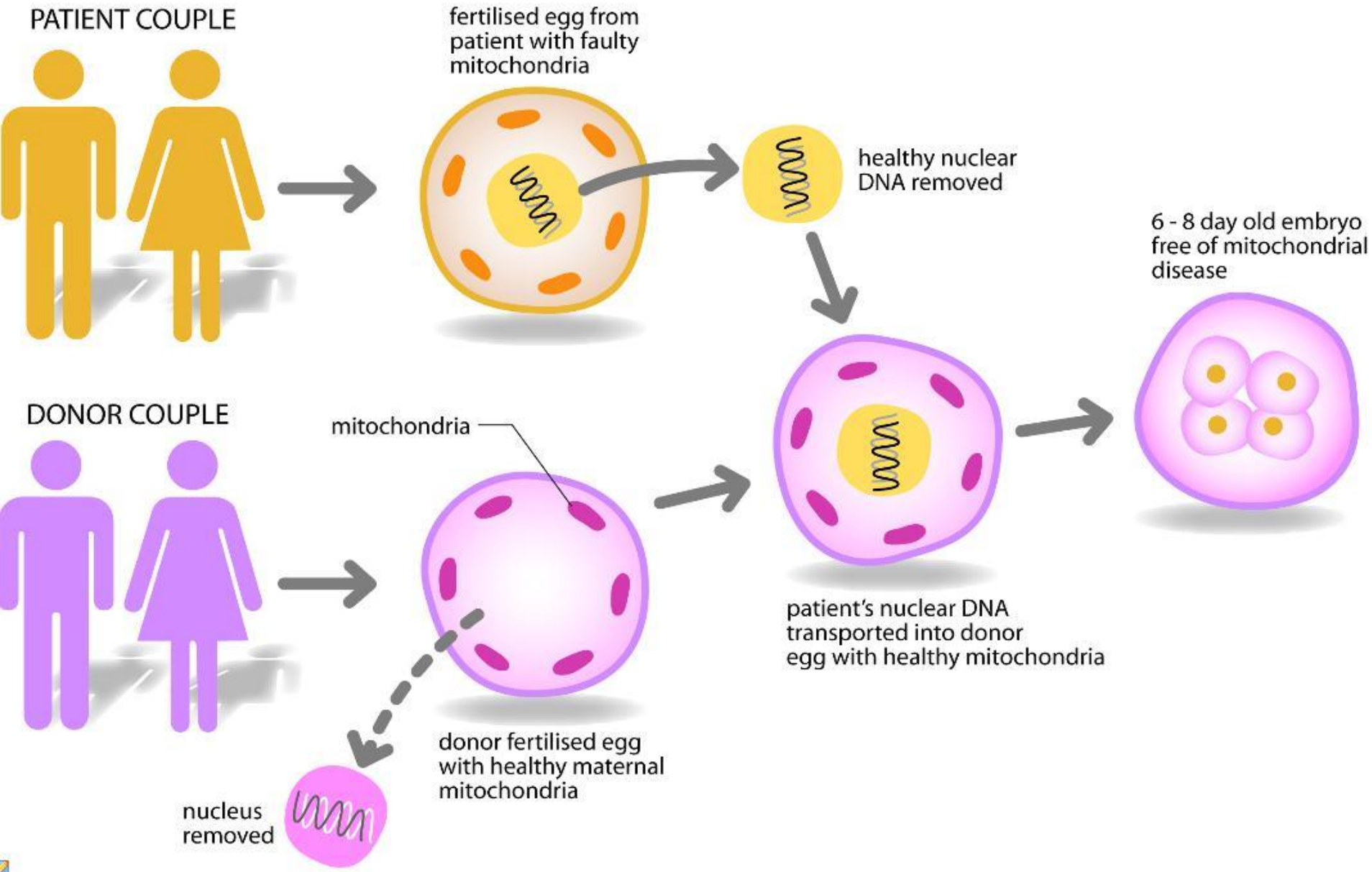


The technique allows to generate gene modified skin required in patients suffering from burns, skin disease, diabetes, etc.



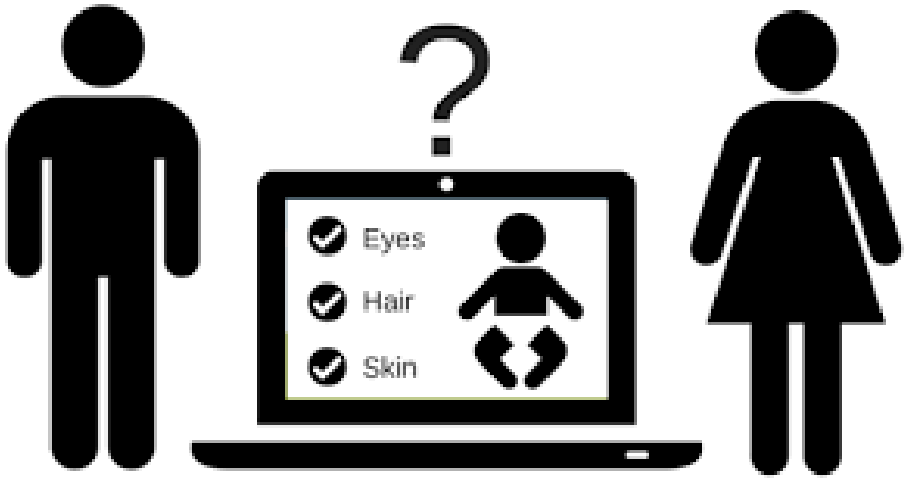
Germline Gene Therapy

Pronuclear transfer in human embryos



Eugenics

- It theoretically allows biotechnology to selectively breed humans with particular properties;
- Even though this branch implies great beneficial potentials - like the eradication of hereditary diseases – wrong use can easily trigger the reverse effect.
- This branch of gene technology is viewed with great interest by the insurance industry and the military.



SOMATIC CELL GENE THERAPY	GERM LINE GENE THERAPY
▪ Therapeutic genes transferred into the **somatic cells.**	▪ Therapeutic genes transferred into the **germ cells.**
▪ Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc.	▪ Eg. Genes introduced into eggs and sperms.
▪ **Will not be inherited** later generations.	▪ **It is heritable** and passed on to later generations.
▪ At present all researches directed to correct genetic defects in somatic cells.	▪ For safety, ethical and technical reasons, it is not being attempted at present.

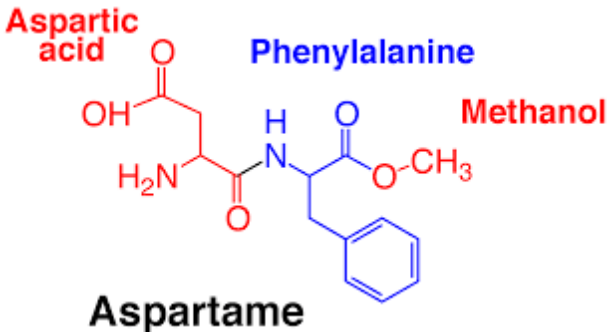
!!! Use germ line gene therapy includes extremely high risks of causing birth defects or even the death of the resulting embryo or fetus.

ECONOMICS –
Who Will Benefit from Biotechnology?

The high- industrialized countries will benefit a lot from biotechnology

The low-developed countries that are directed to produce agricultural and other raw will lose extremely.

Biotechnology brought major changes to the **sugar industry**.



200 % sweeter than normal sugar; not have any calories. Used as sugar synthetical substitute

Sugar producing countries like the Philippines suffered tremendously.

Widely used in food and soft drink industry.

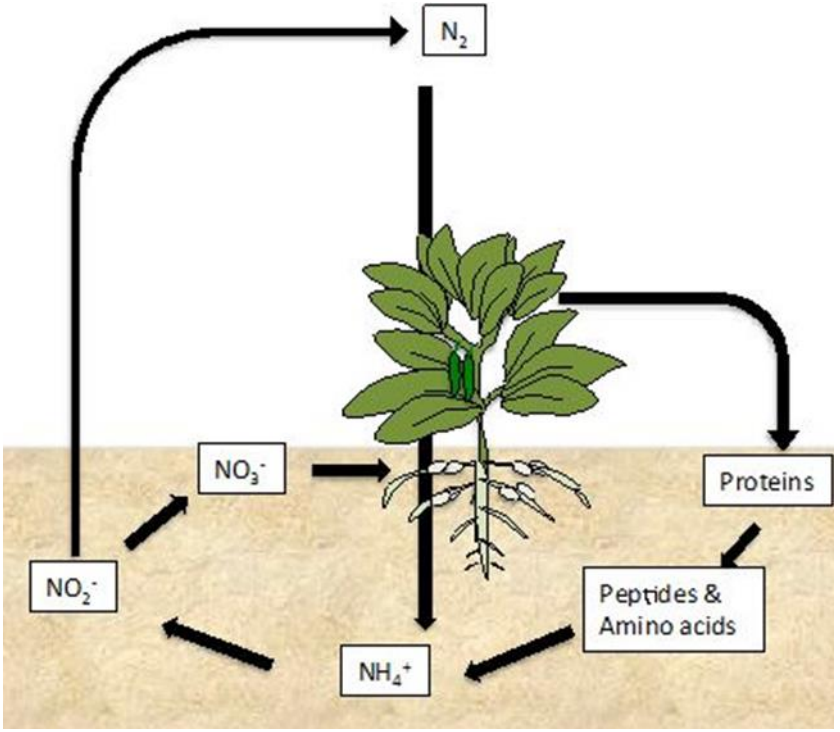
High Fructose Corn Syrup is obtained from corn starch; it is 150% sweeter than conventional sugar.

Following the current agricultural concept of the EU such substitutes are not approved to use.

Rice production is improved by Nitrogen-fixing *Cyanobacteria*

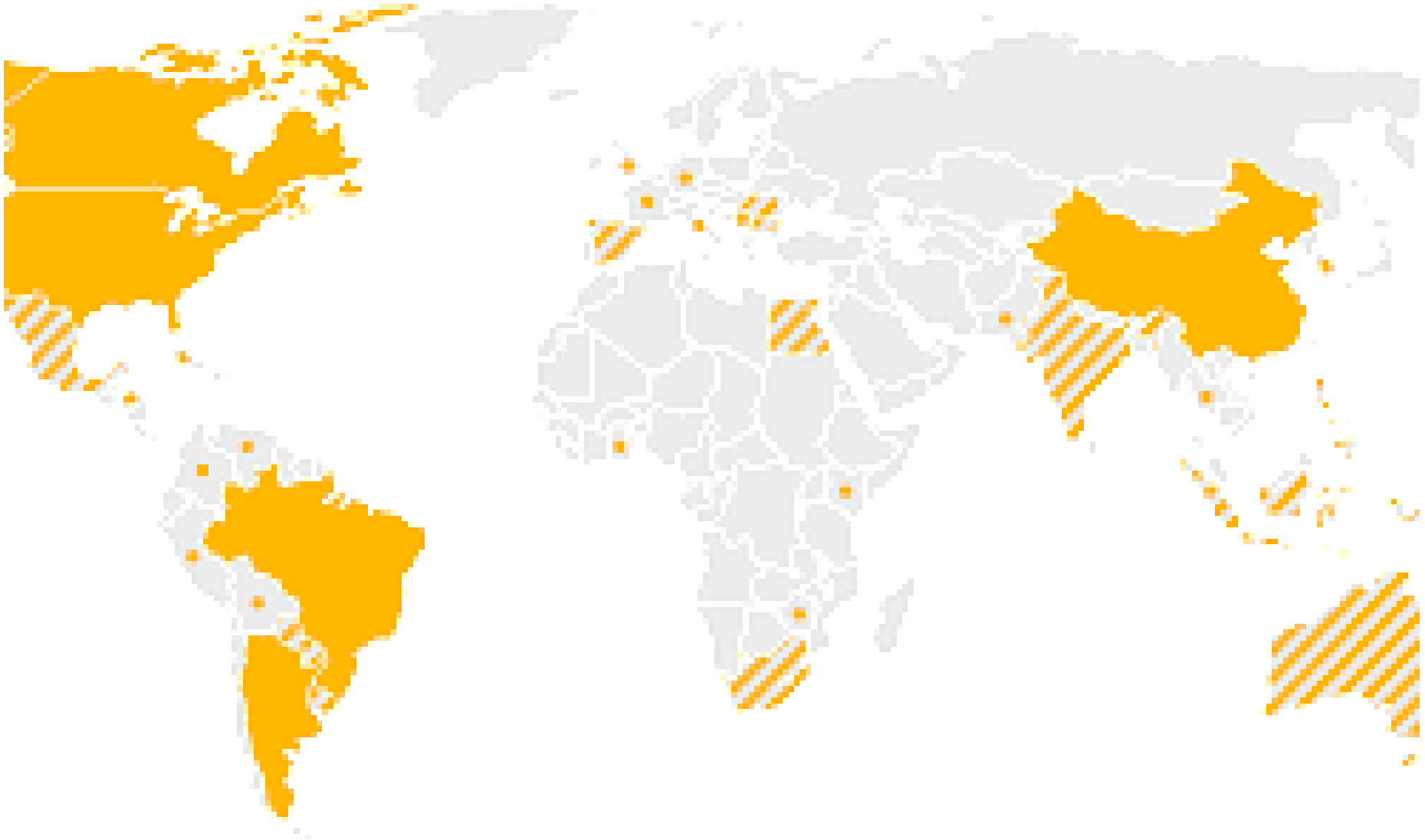
Nitrogen fixation-convert atmospheric nitrogen (N_2) into ammonium (NH_4^+) which can be used to make organic compounds like amino acids.

- Nitrogen-fixing cyanobacteria are essential to maintaining the fertility of semi-aquatic environments like rice paddies.



India, Indonesia greatly increased rice yields using modified Cyanobacteria.

Pakistan suffered enormously.



Yellow is the area with the most use of GM-technology

Research and development benefits greatly from biotechnology, amongst other effects it:

a) creates new skills and new jobs;

b) improved quality of life due to progresses in health care;

c) increased yields in agriculture;

d) missing raw materials in old world can be produced by implementing GM-microorganisms that facilitate cost-effective production of these materials.

Carlsberg Group

It encourages its farmers to grow raw materials that can be used in the textile and paper industry.


Thank You For
Attention