Biotechnology
Science, Technology and Society

What is Biotechnology?

- It is the manipulation of *living organisms* or *parts of living organisms* to make products useful to humans

- It deals with the manipulation of the genes of organisms to *alter* their *behaviour*, *characteristics*, or *value*

- Cell and gene technology used to *produce new characteristics* in *plants* and *animals*

What is Biotechnology?

Table 1.1 Some selected definitions of biotechnology
A collective noun for the application of biological organisms, systems or processes to manufacturing and service industries.
The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application capabilities of microorganisms, cultured tissue cells and parts thereof.
A technology using biological phenomena for copying and manufacturing various kinds of useful substances.
The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.
The science of the production processes based on the action of microorganisms and their active components and of production processes involving the use of cells and tissues from higher organisms. Medical technology, agriculture and traditional crop breeding are not generally regarded as biotechnology.
Really no more than a name given to a set of techniques and processes.
The use of living organisms and their components in agriculture, food and other industrial processes.
The deciphering and use of biological knowledge.
The application of our knowledge and understanding of biology to meet practical needs.

Timeline of Biotechnology

Science, Technology and Society

Timeline of Biotechnology

8000 B.C.E

Domestication of plants and animal

4000 B.C.E

Egyptians master the art of wine making

Timeline of Biotechnology

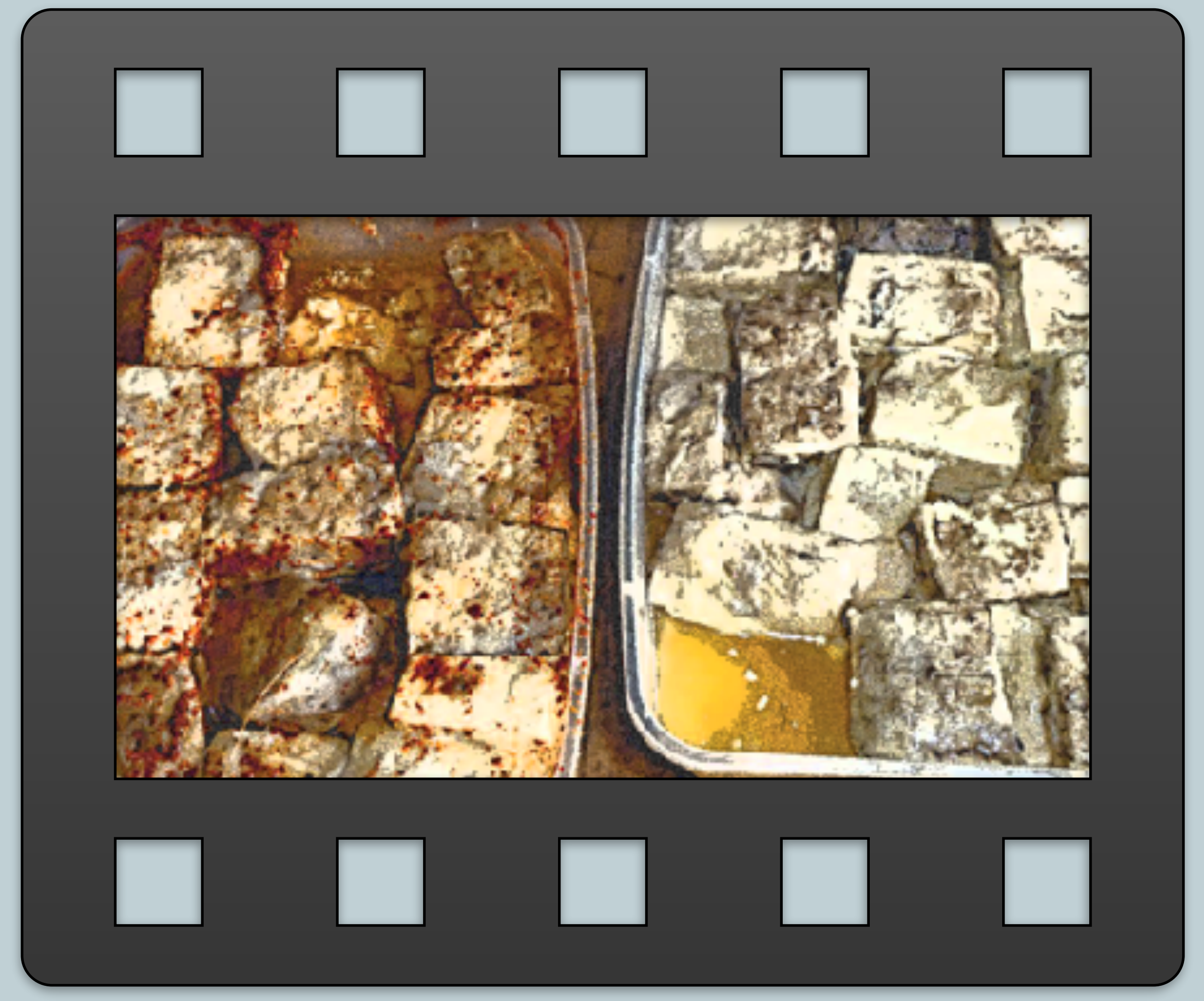
2000 B.C.E

Egyptians used yeast to make bread

2000 B.C.E

Egyptians and Sumerians learned brewing and cheese making

Timeline of Biotechnology



500 B.C.E

Mouldy soybean curds used to treat boils

300 B.C.E

Greeks develop grafting techniques

Timeline of Biotechnology

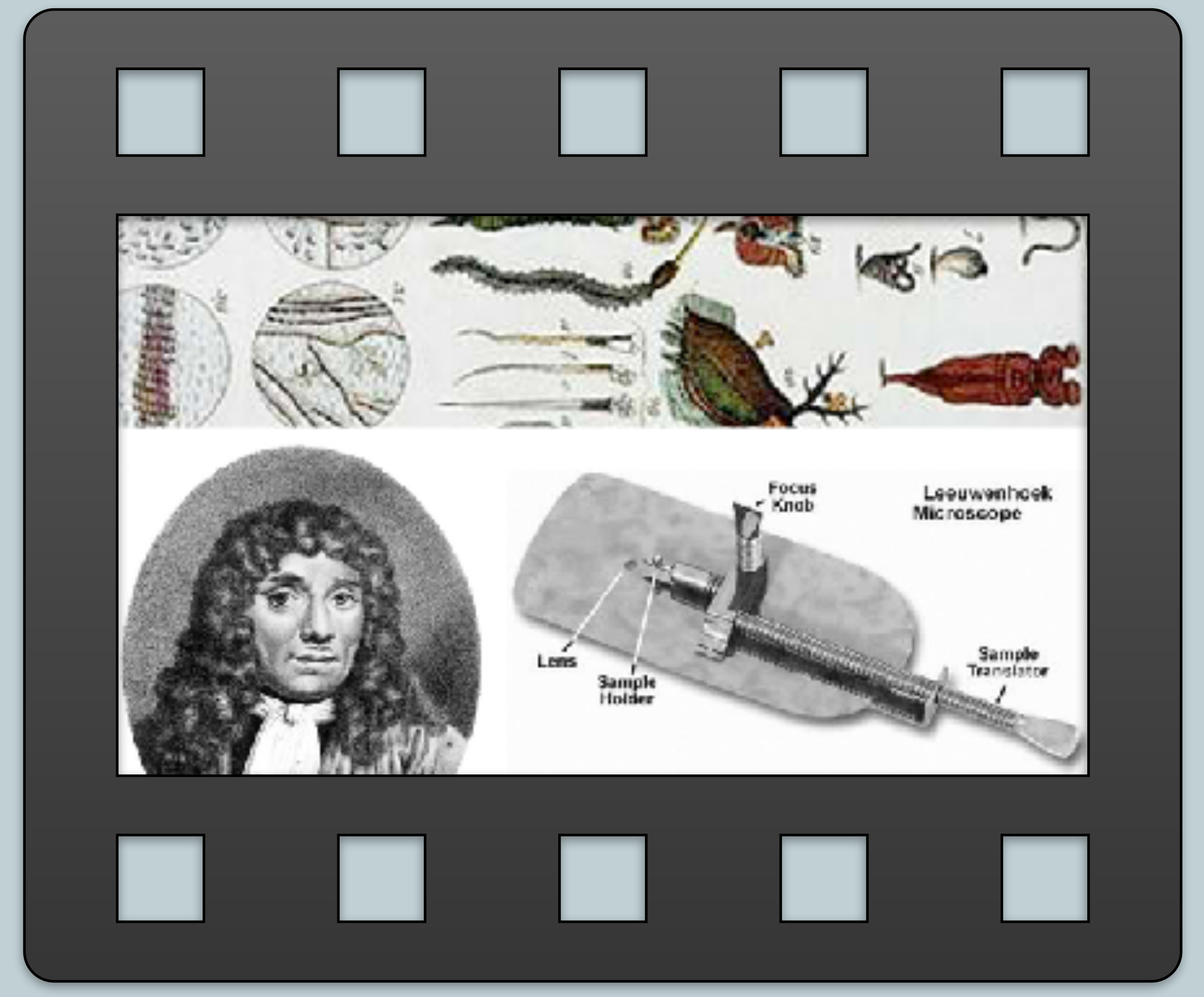
100 C.E

Powdered chrysanthemums

1663

Robert Hooke described the cell

Timeline of Biotechnology



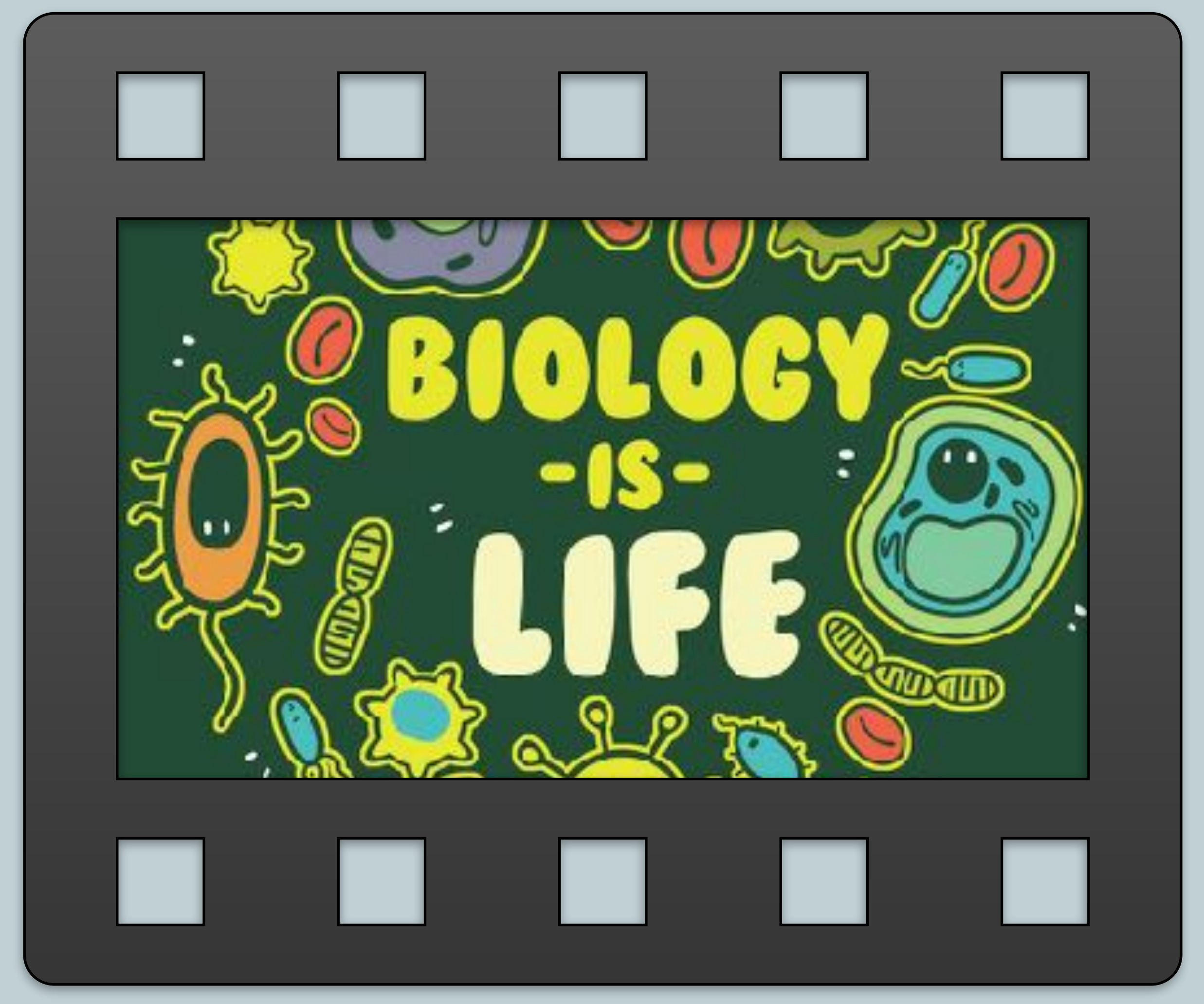
1675

Anton van Leeuwenhoek discovers protozoa and bacteria

1797

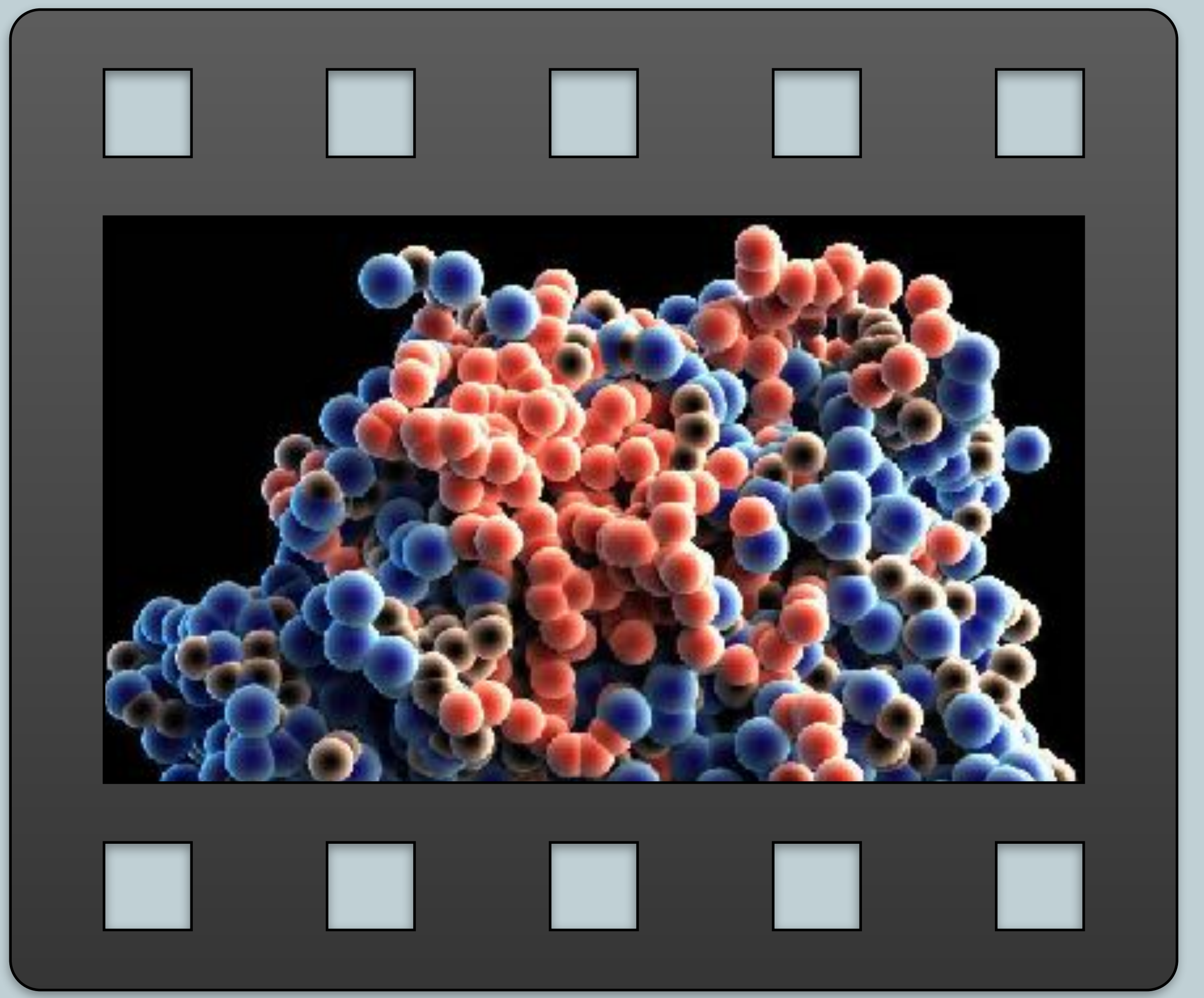
Edward Jenner created the cowpox vaccine

Timeline of Biotechnology



1802

"Biology" first appears



1830

Proteins are discovered

Timeline of Biotechnology

1855

Escherichia coli is discovered by Theodor Escherich

1857

Fermentation and Germ Theory

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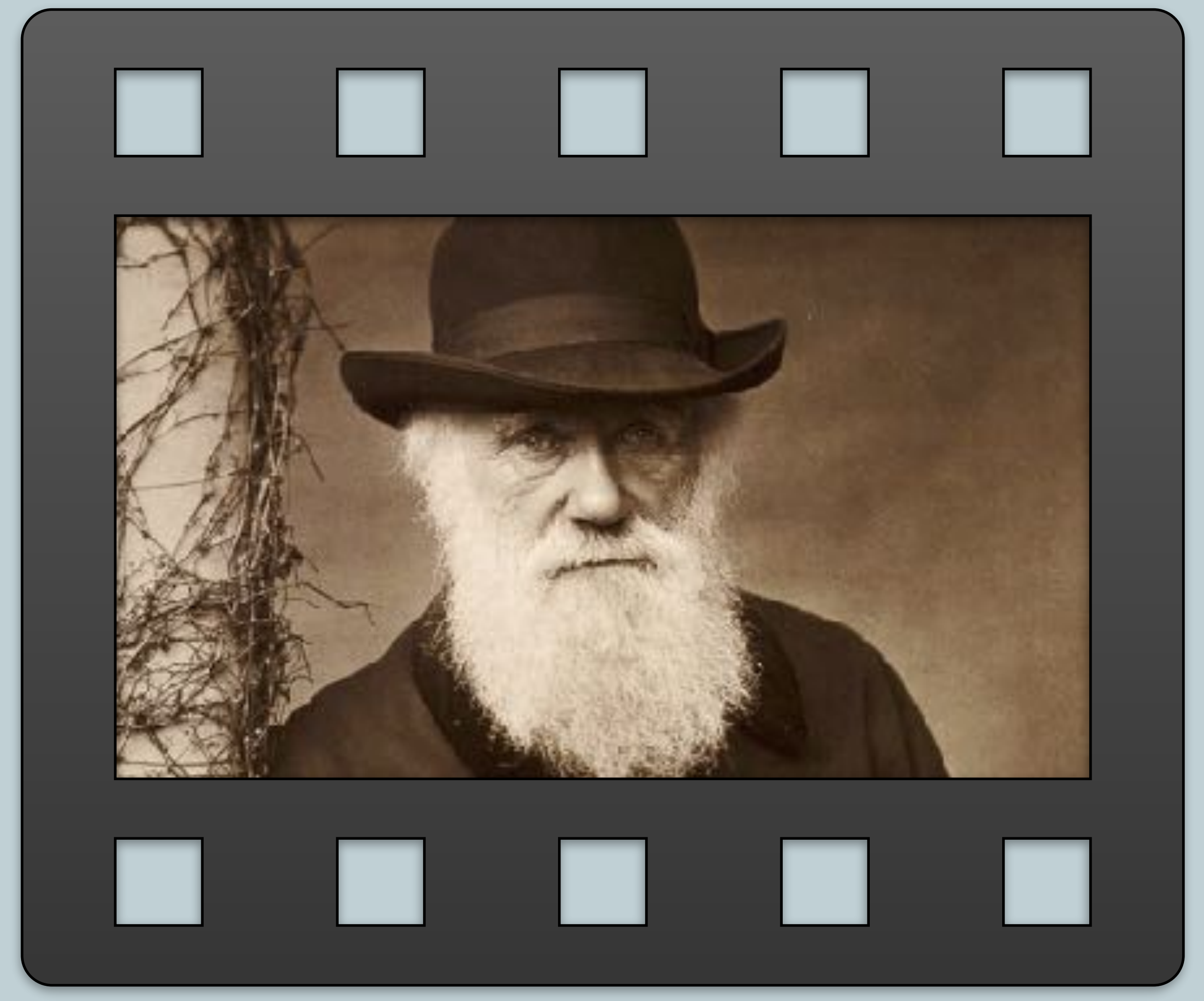

1859

Charles Darwin published the Theory of Evolution by Natural Selection

1861

Louis Pasteur develops pasteurisation

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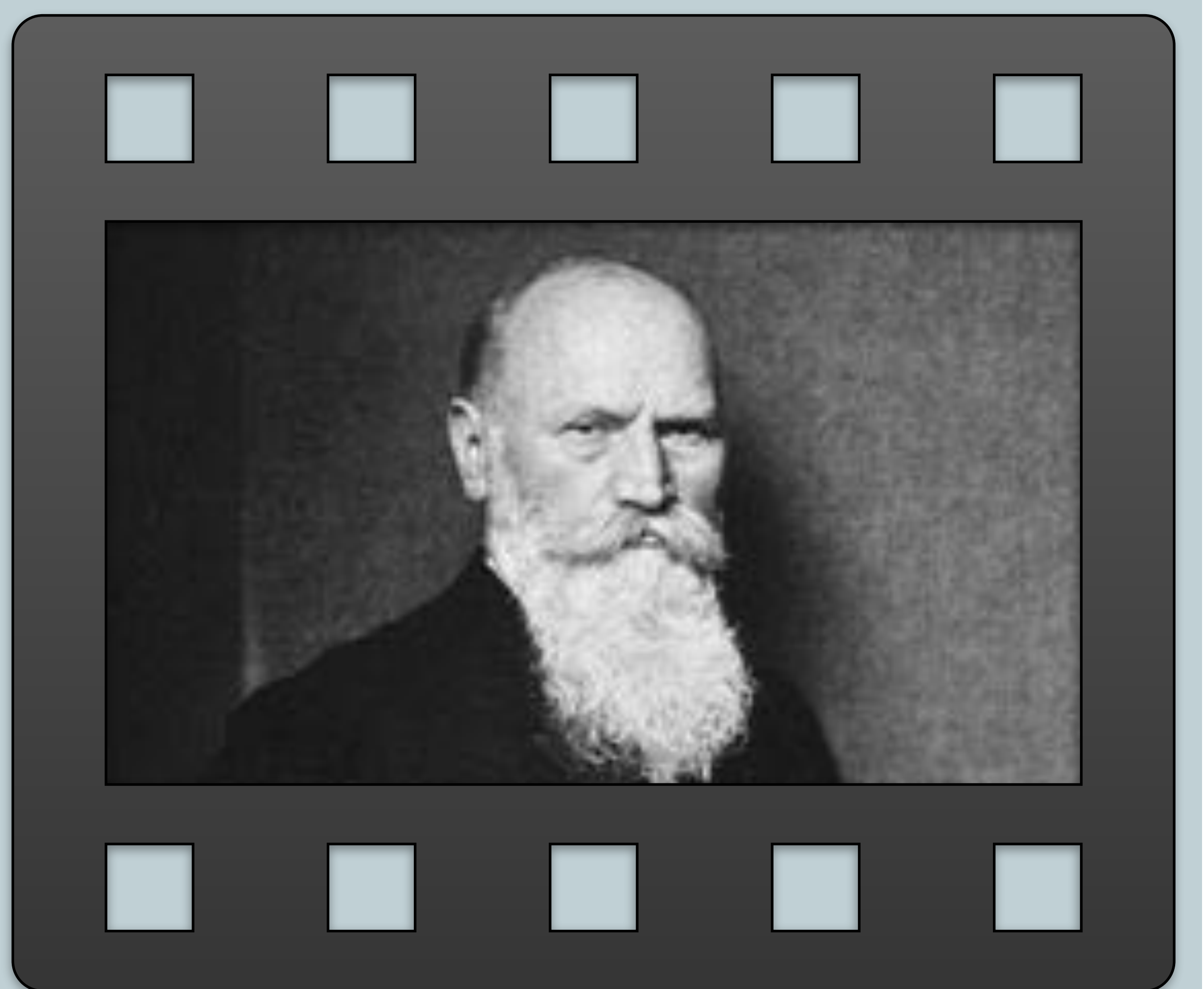
1865

Gregor Mendel and Laws of Inheritance



1888

Heinrich Wilhelm Gottfried Waldeyer discovered the chromosome

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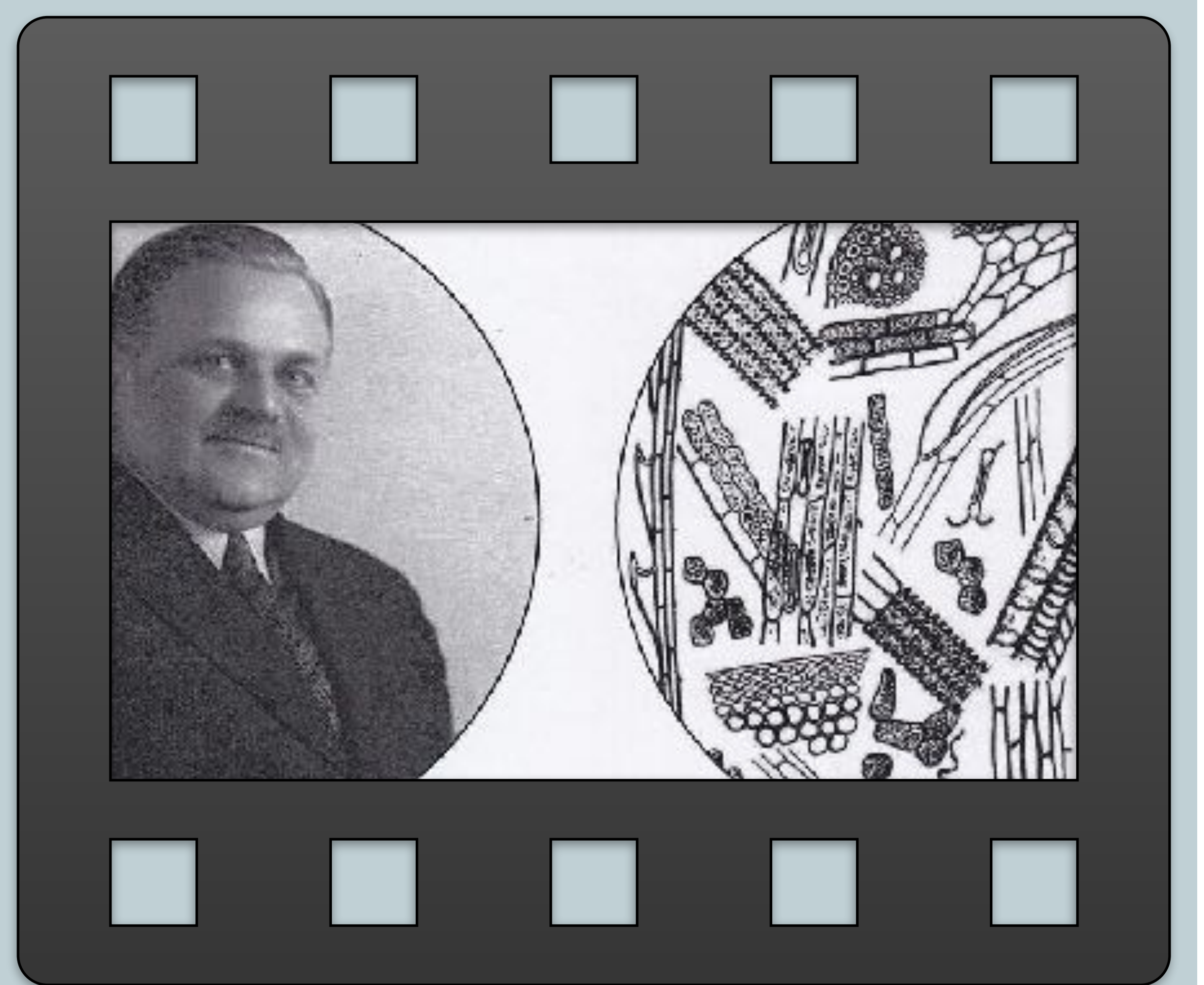
1915

Bacteriophages were discovered



1919

“Biotechnology” was introduced by Károly Ereky

Timeline of Biotechnology

1922

Dr. Frederick Banting and Charles Best discovered insulin

1927

Herman Muller - radiation causes defects in chromosomes

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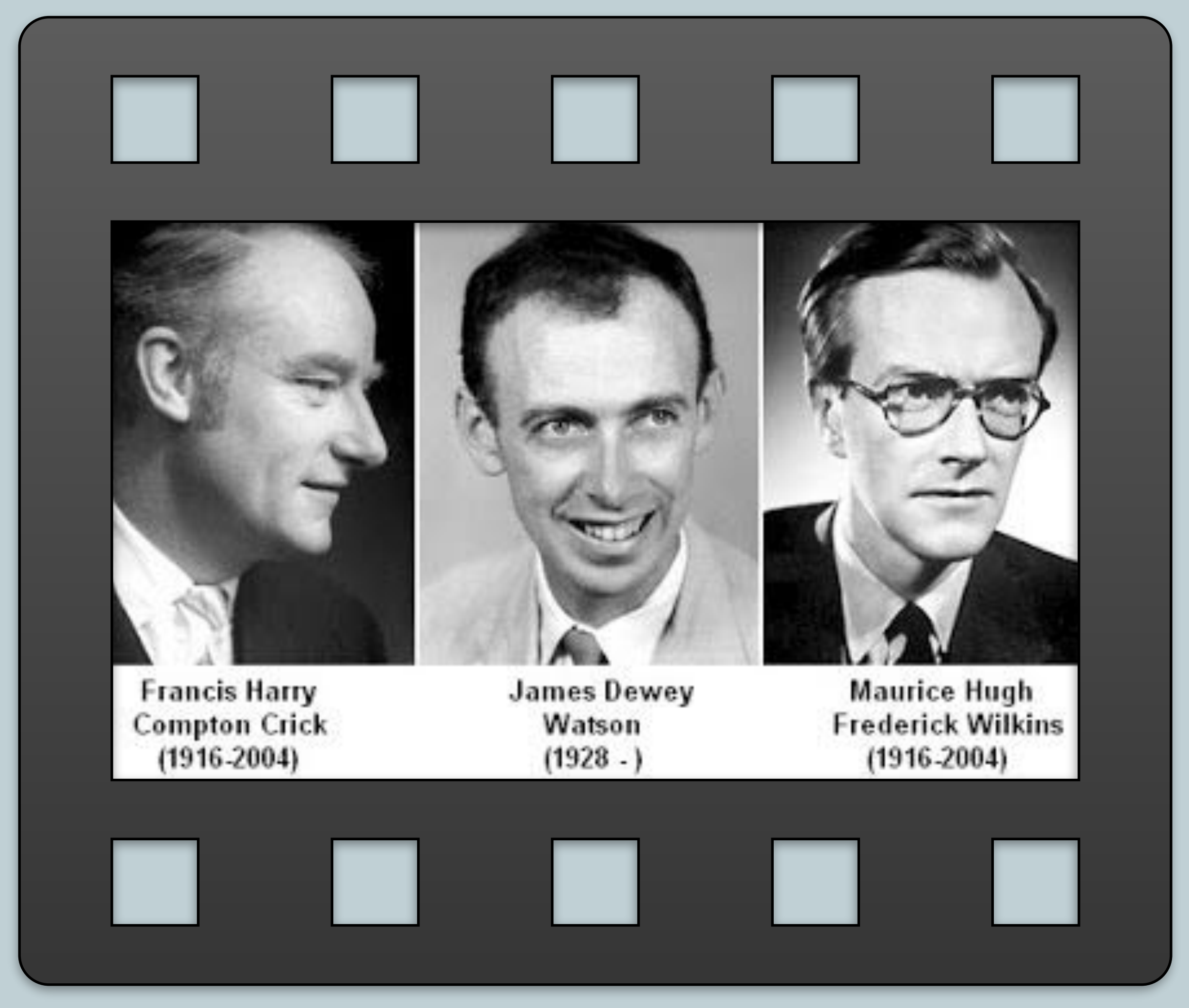
1928

Alexander Fleming and antibiotic penicillin

1944

Oswald Avery, Colin MacLeod and Maclyn McCarty proved that the DNA carries the genetic information

Timeline of Biotechnology



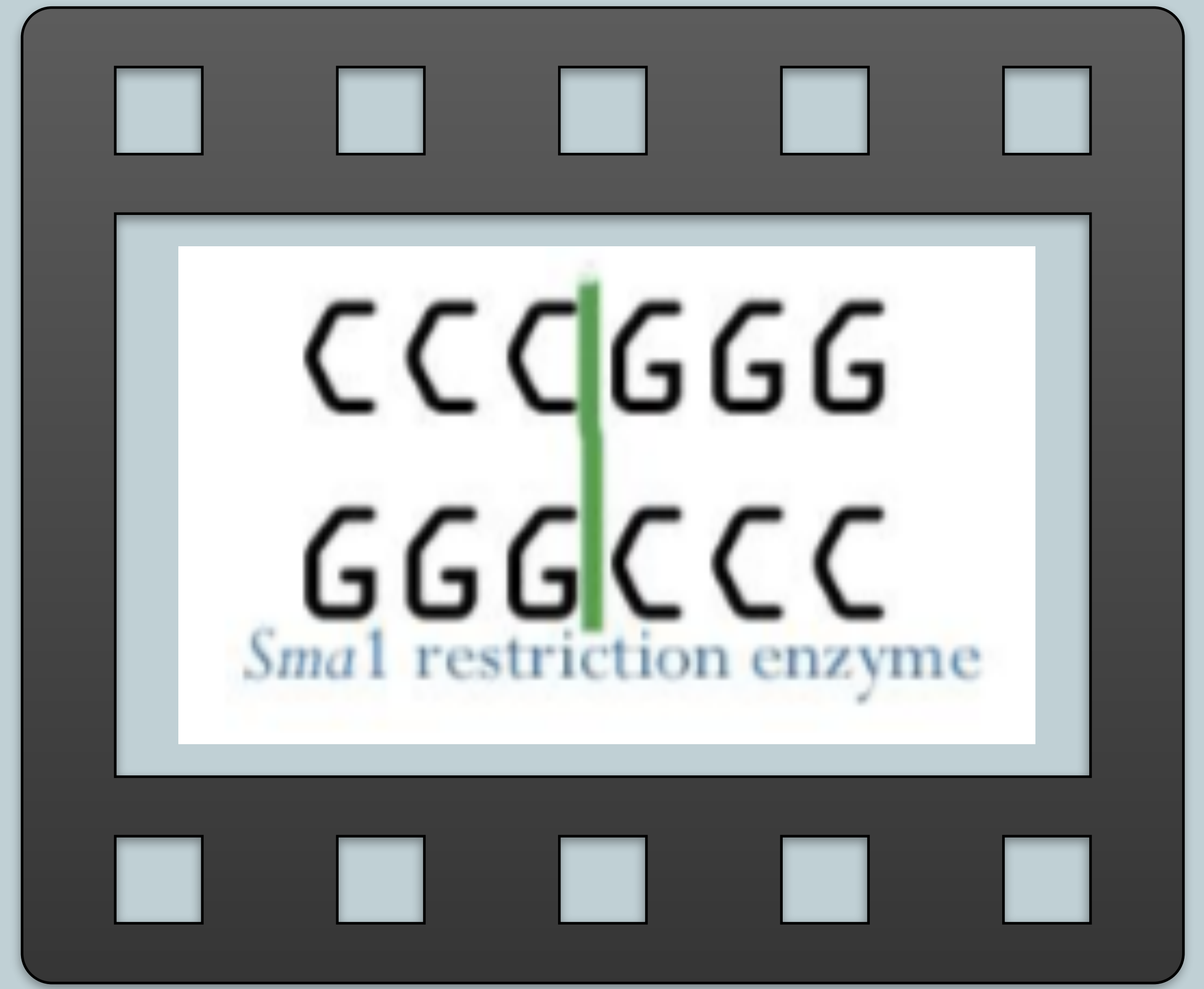
1953

Watson, Crick and Wilkins described the 3d Model of DNA

1966

The genetic code for DNA is cracked

Timeline of Biotechnology



1971

The first complete synthesis of gene occurs

1973

Stanley Cohen and Herbert Boyer perfected genetic engineering techniques

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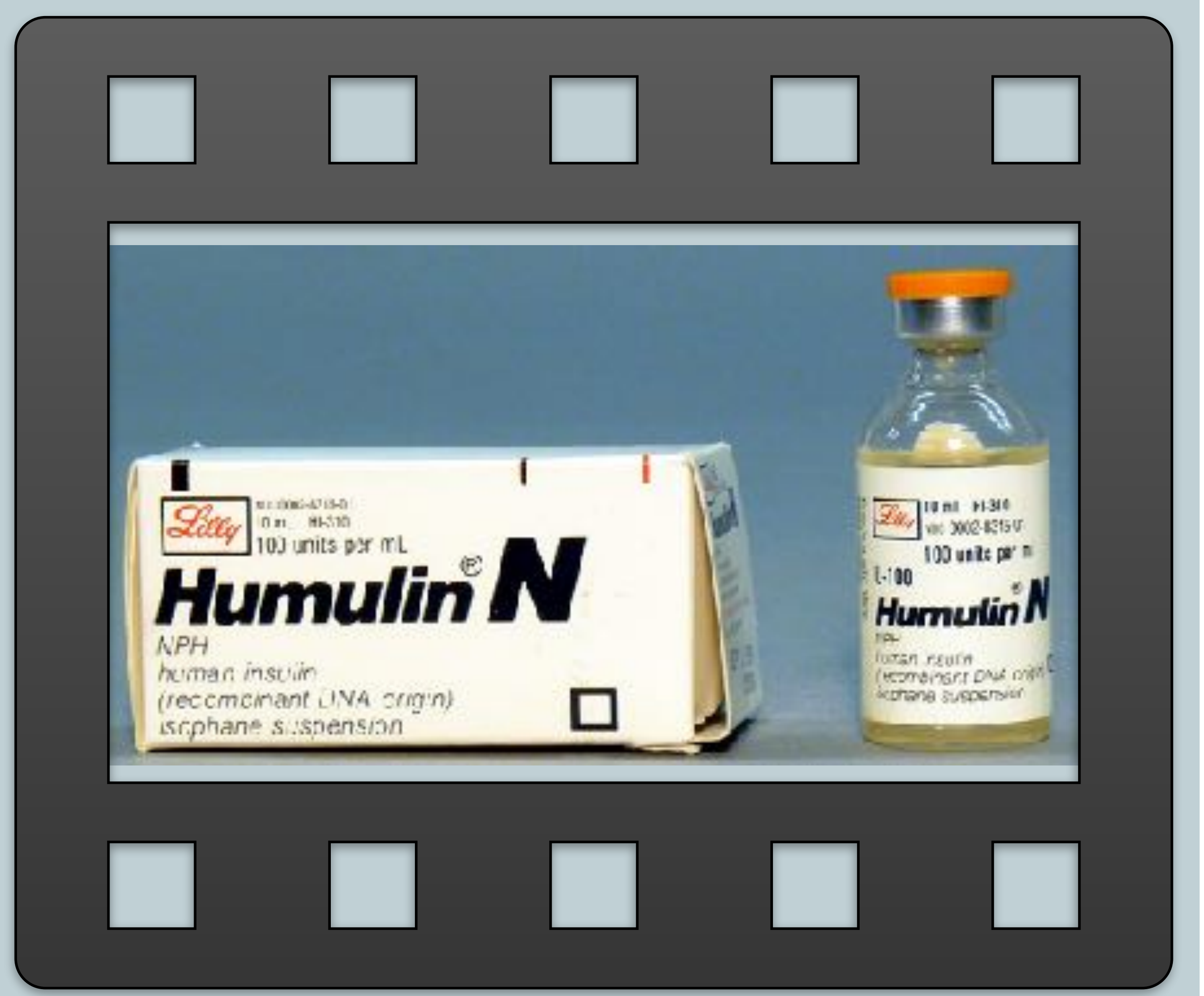
1975

George Kohler and Cesar Milstein developed the technology to produce monoclonal antibodies



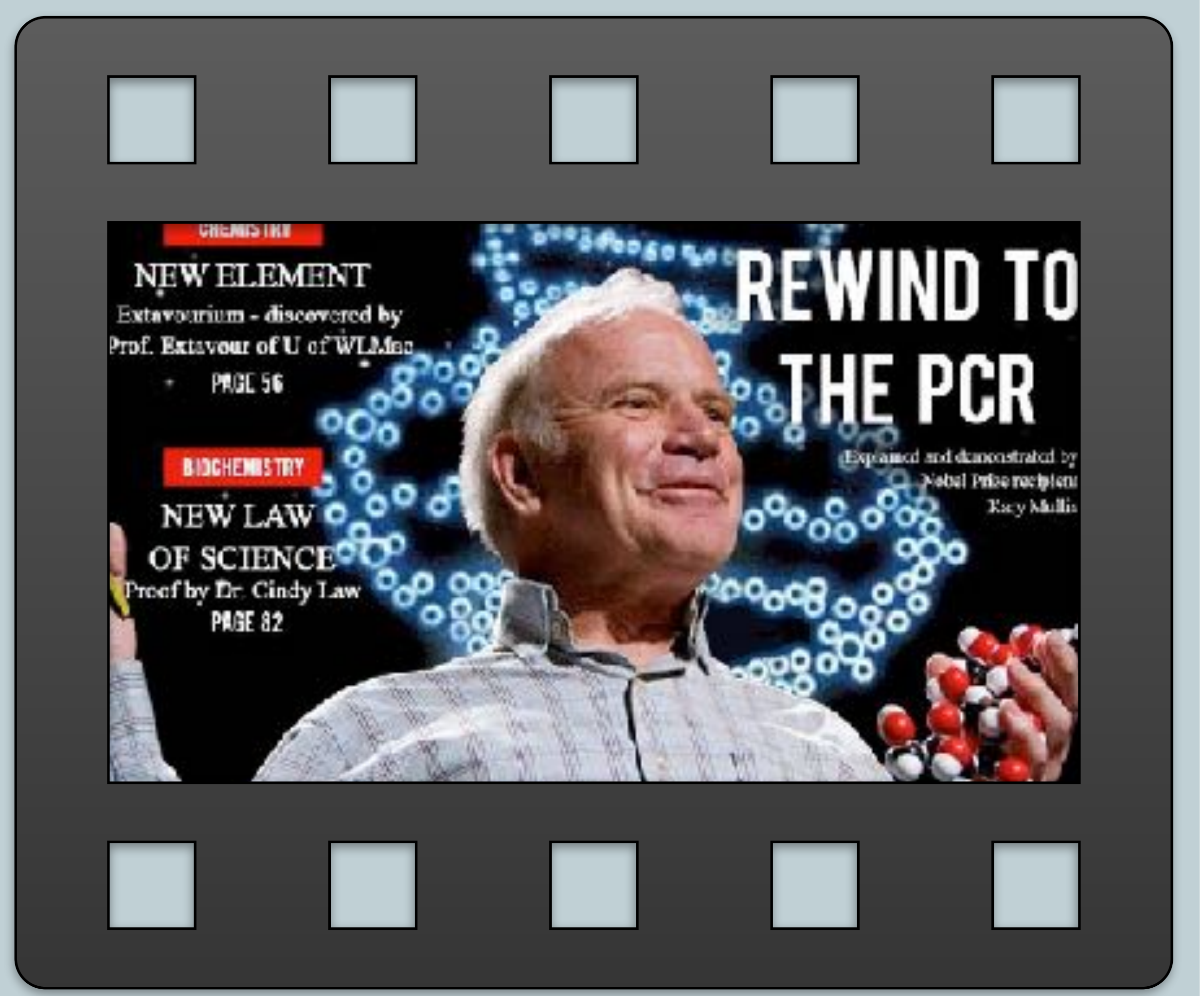
1982

First FDA approved human insulin was produced

Timeline of Biotechnology

1981

First transgenic animals are produced



1983

Polymerase Chain Reaction (PCR) technique by Kary Mullis

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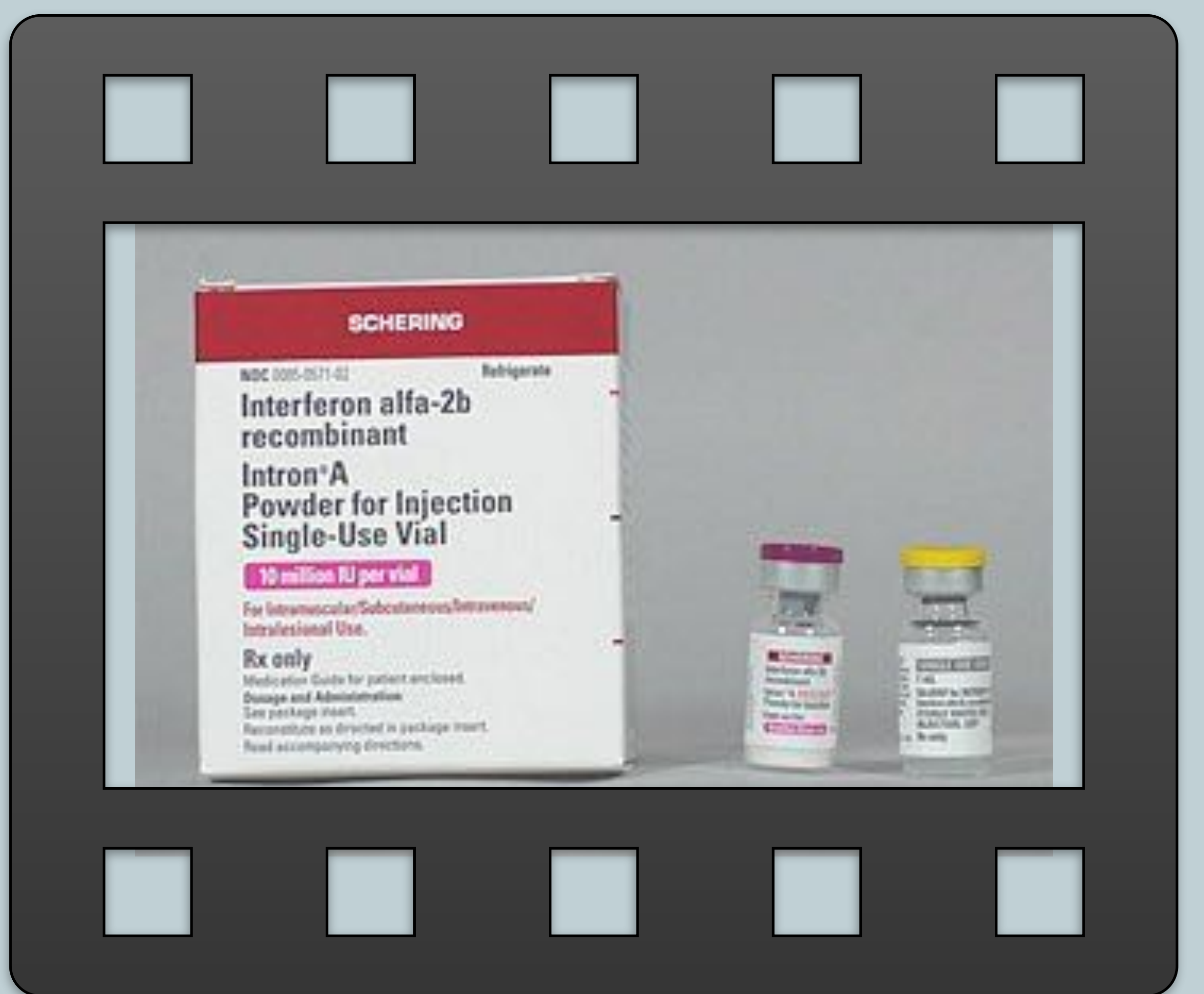
1986

First recombinant vaccine : Hepatitis B



1986

First anti cancer drug : Interferon

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1987

GMO : Virus-resistant tomatoes

1994

First GMO product was sold in the U.S.

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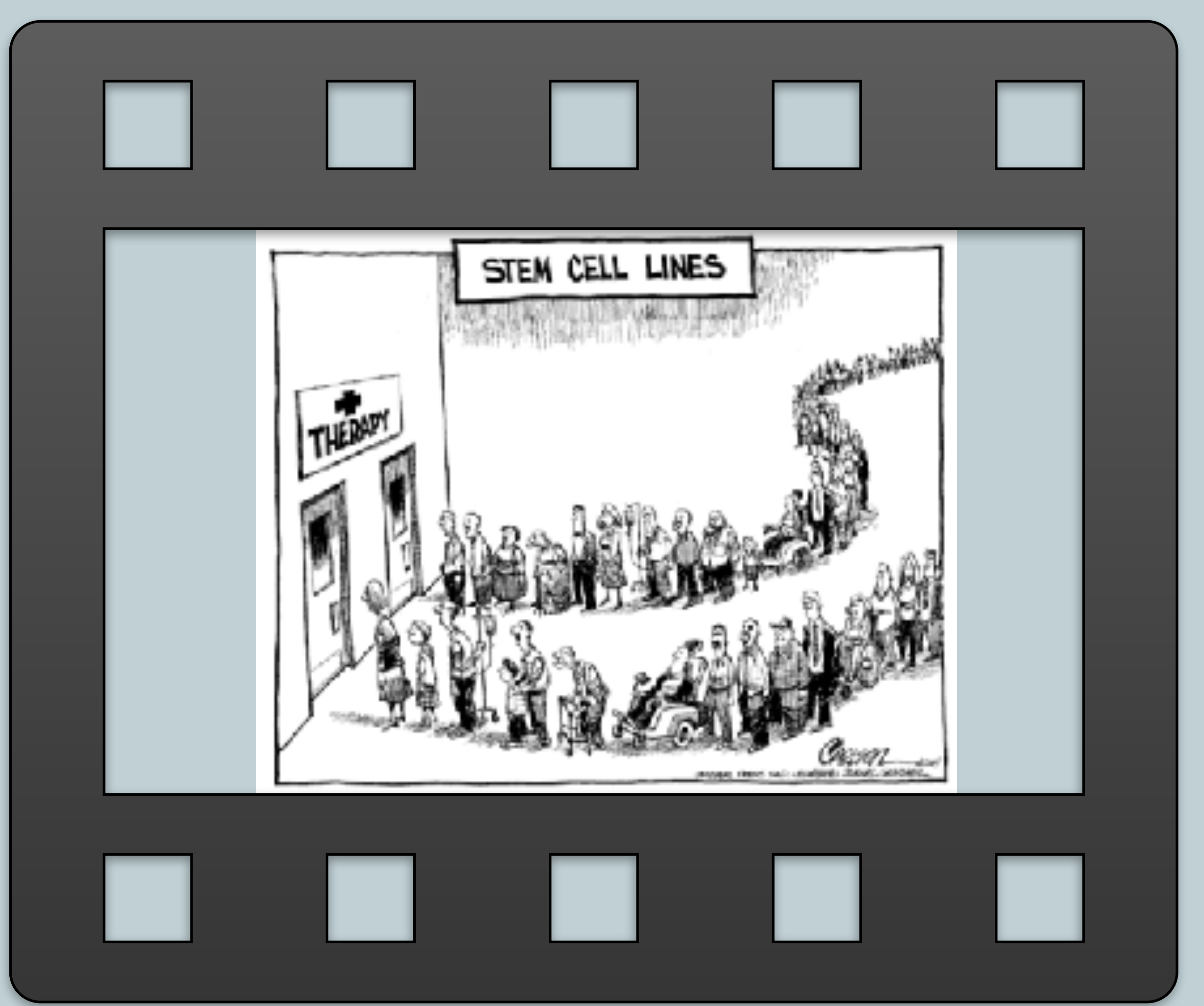
1997

The first cloned animal from an adult cell : Dolly



1998

Human Embryonic Stem Cell Lines are established

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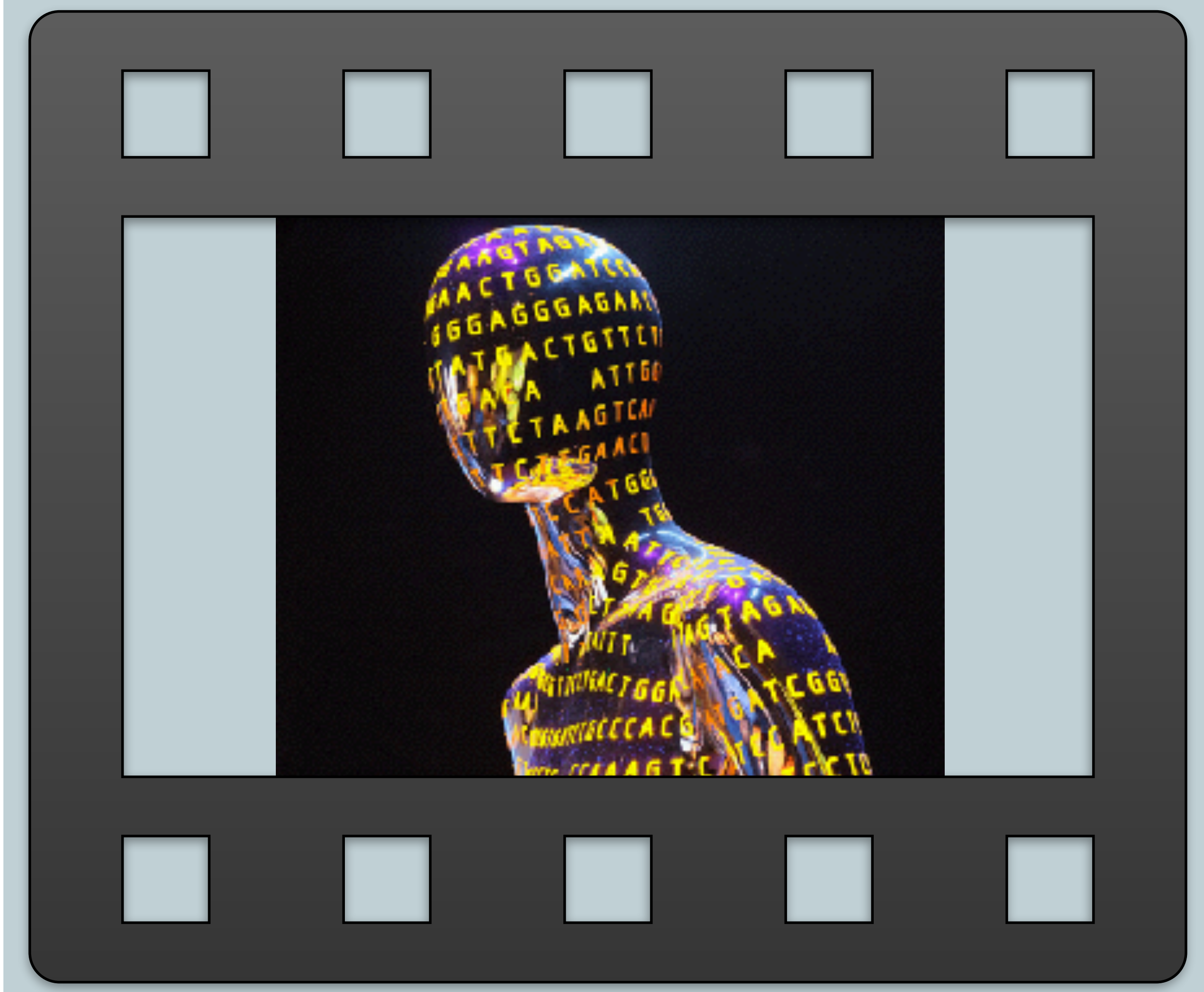


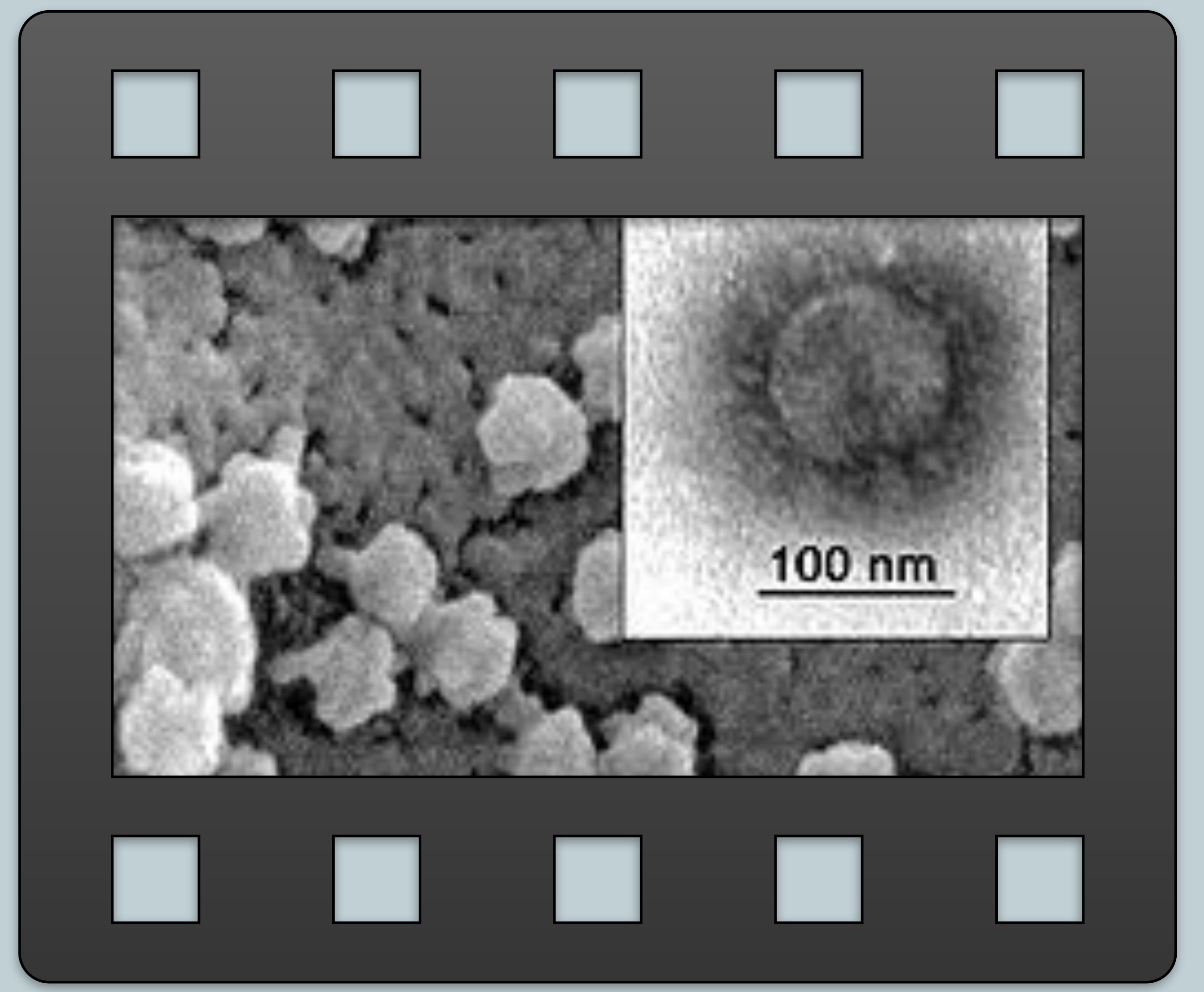
1999

The Human Genome Project is launched

2002

Draft version of THGP is published

Timeline of Biotechnology



2003

Severe Acute Respiratory Syndrome (SARS) virus is sequenced

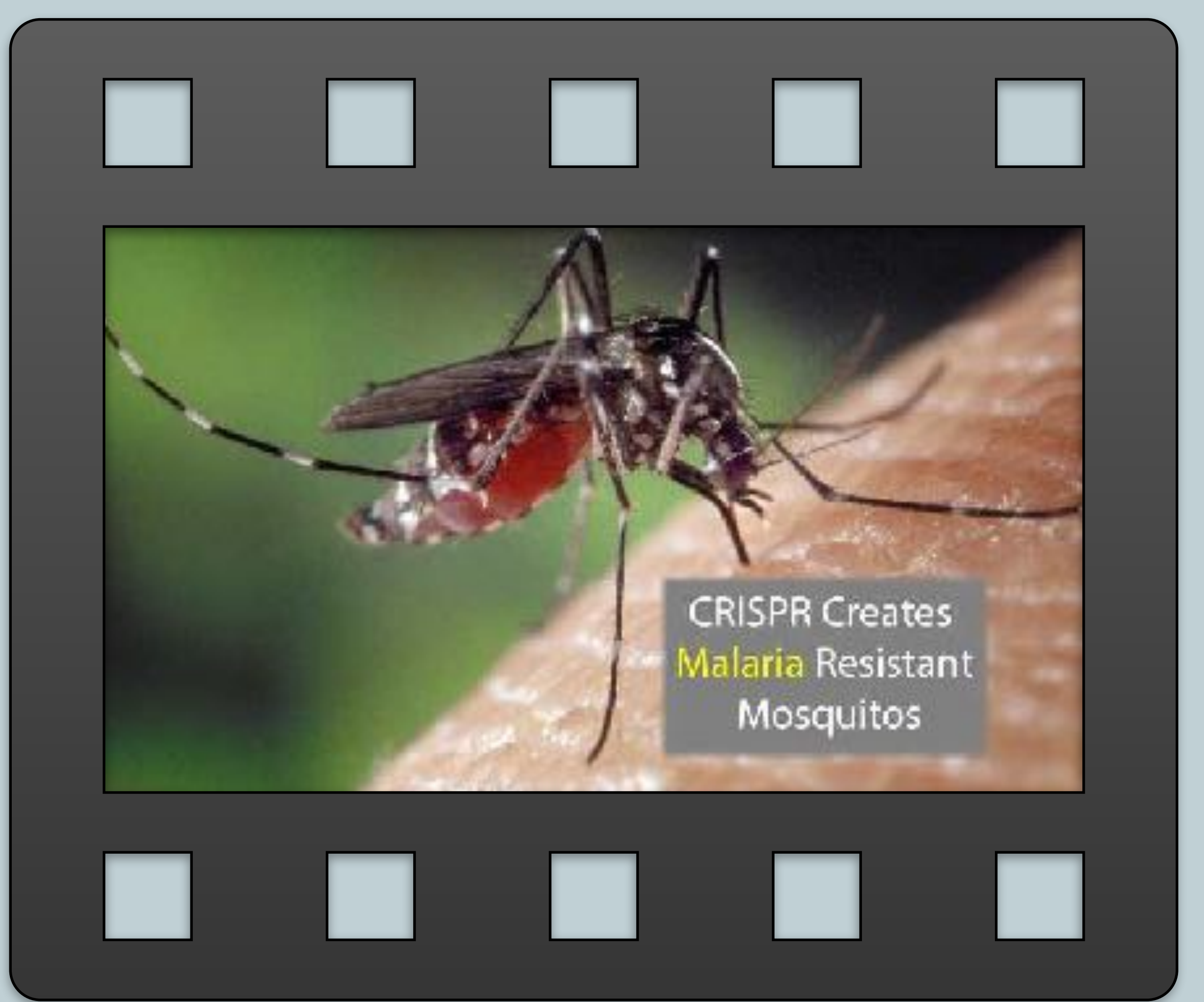
2004

First cloned pet

Timeline of Biotechnology

2006

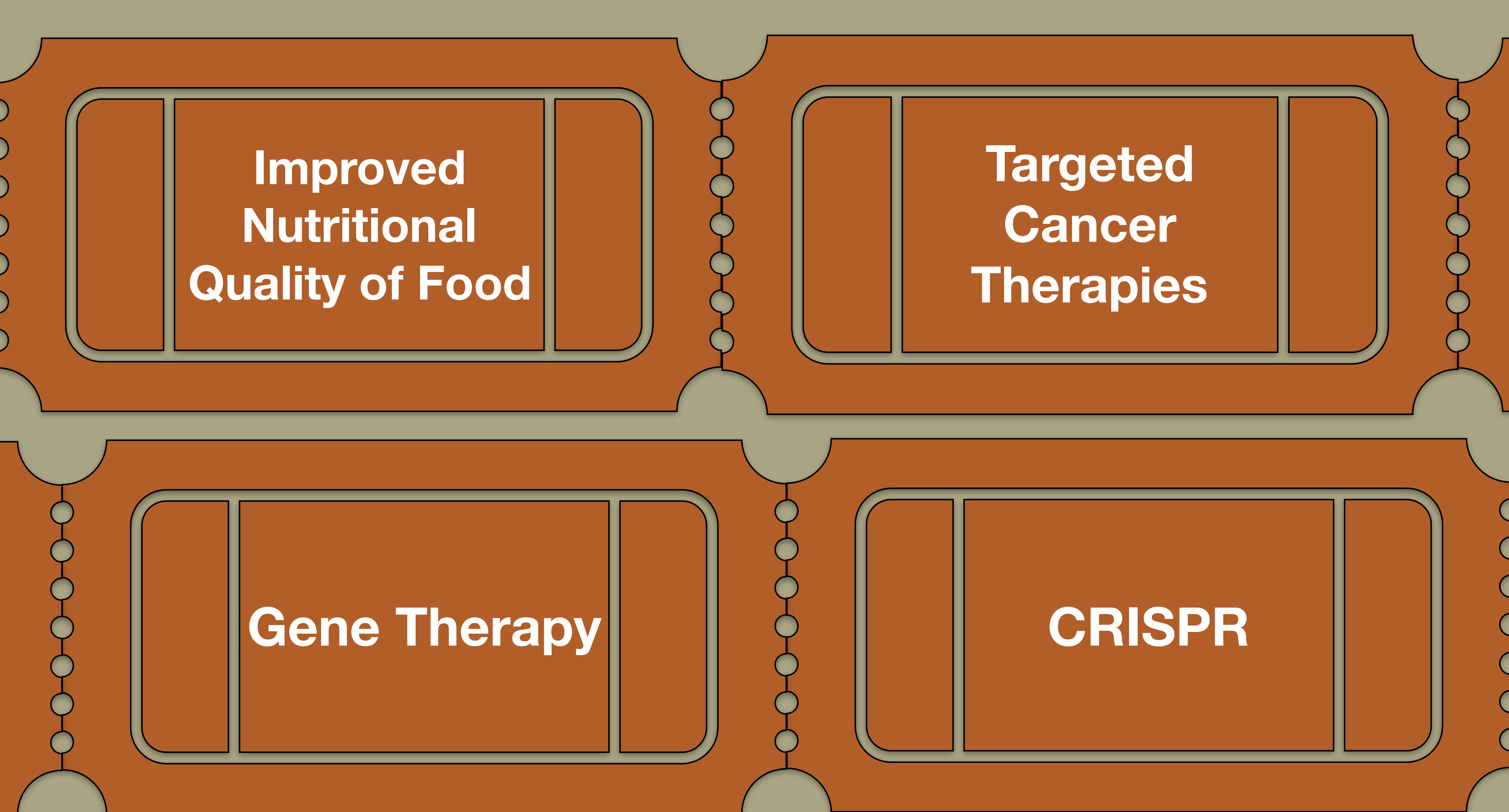
Recombinant vaccine against Human Papillomavirus (HPV)



2010

Malaria-resistant mosquitoes

Recent Breakthroughs



Types of Biotechnology

- Microbial Biotechnology
- Agricultural Biotechnology
- Animal Biotechnology
- Forensic Biotechnology
- Bioremediation
- Aquatic Biotechnology
- Medical Biotechnology

Microbial Biotechnology

- Manipulation of microorganisms such as yeast and bacteria
  - Create better enzymes
  - More efficient decontamination processes for industrial waste product removal
  - Used to clone and produce large amounts of important proteins used in human medicine

Agricultural Biotechnology

- Plants more environmentally friendly that yield more per acre (genetically engineered)
- Resistance to diseases and insects
- Foods with higher protein or vitamin content
- Drugs developed and grown as plant products
- These better plants ultimately reduce production costs to help feed the growing world population

Animal Biotechnology

- Animals as a source of medically valuable proteins
  - Antibodies
  - Transgenic animals
- Animals as important models in basic research
  - Gene "knockout" experiments
  - Design and testing of drugs and genetic therapies
- Animal cloning

Forensic Biotechnology

- **DNA fingerprinting**
  - Inclusion or exclusion of a person from suspicion
  - Paternity cases
  - Identification of human remains
  - Endangered species
  - Tracking and confirmation of the spread of disease

Bioremediation

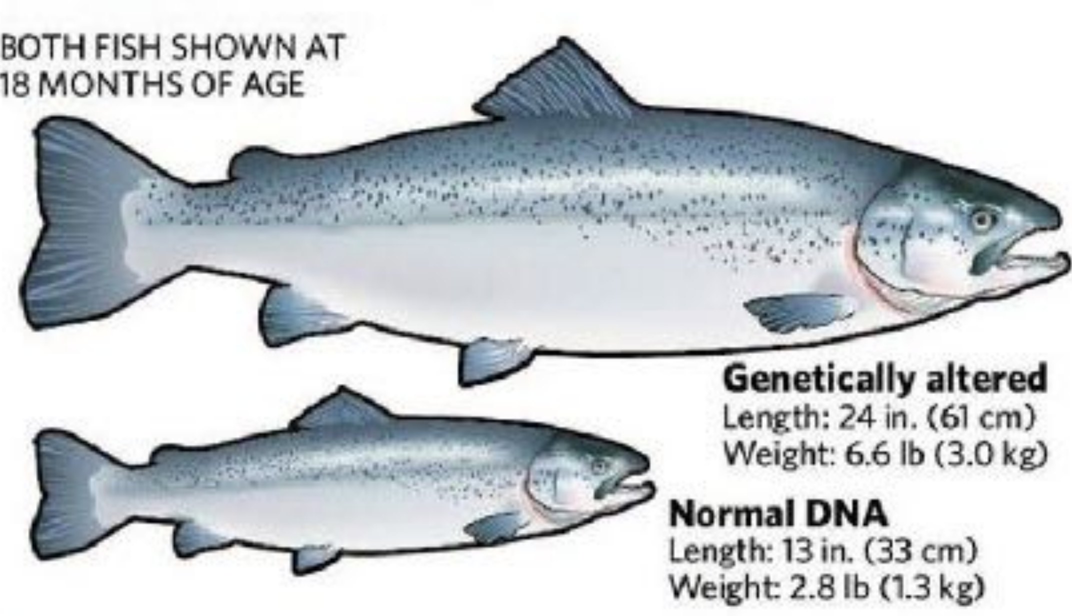
- The use of biotechnology to process and degrade a variety of natural and manmade substances
  - Particularly those that contribute to environmental pollution
- Example – stimulated growth of bacteria that degrade components in crude oil
  - 1989 Exxon Valdez oil spill in Alaska
  - 2010 Deep Water Horizon spill

Bioremediation

- **Bioremediation** – adding nutrients to stimulate growth of bacteria to clean up oil spill
  - *Alcanivorax borkumensis*

Aquatic Biotechnology

- **Aquaculture**
  - Raising finfish or shellfish in controlled conditions for use as food sources
    - 50% of all fish consumed by humans worldwide
- **Genetic engineering**
  - Disease-resistant strains of oysters
  - Vaccines against viruses that infect salmon and other finfish
  - Transgenic salmon that overproduce growth hormone
- **Bioprospecting**
  - Rich and valuable sources of new genes, proteins and metabolic processes with important applications for human benefits
    - Marine plankton and snails found to be rich sources of antitumor and anticancer



Medical Biotechnology

- Involved with the whole spectrum of human medicine
  - Preventive medicine
  - Diagnosis of health and illness
  - Treatment of human diseases
- New information from Human Genome Project
  - Gene therapy
  - Stem cell technologies

Pros and Cons

Pros

It can improve health and reduce hunger simultaneously

It creates flexibility within the food chain

☑ Pros

It offers medical advancement opportunities

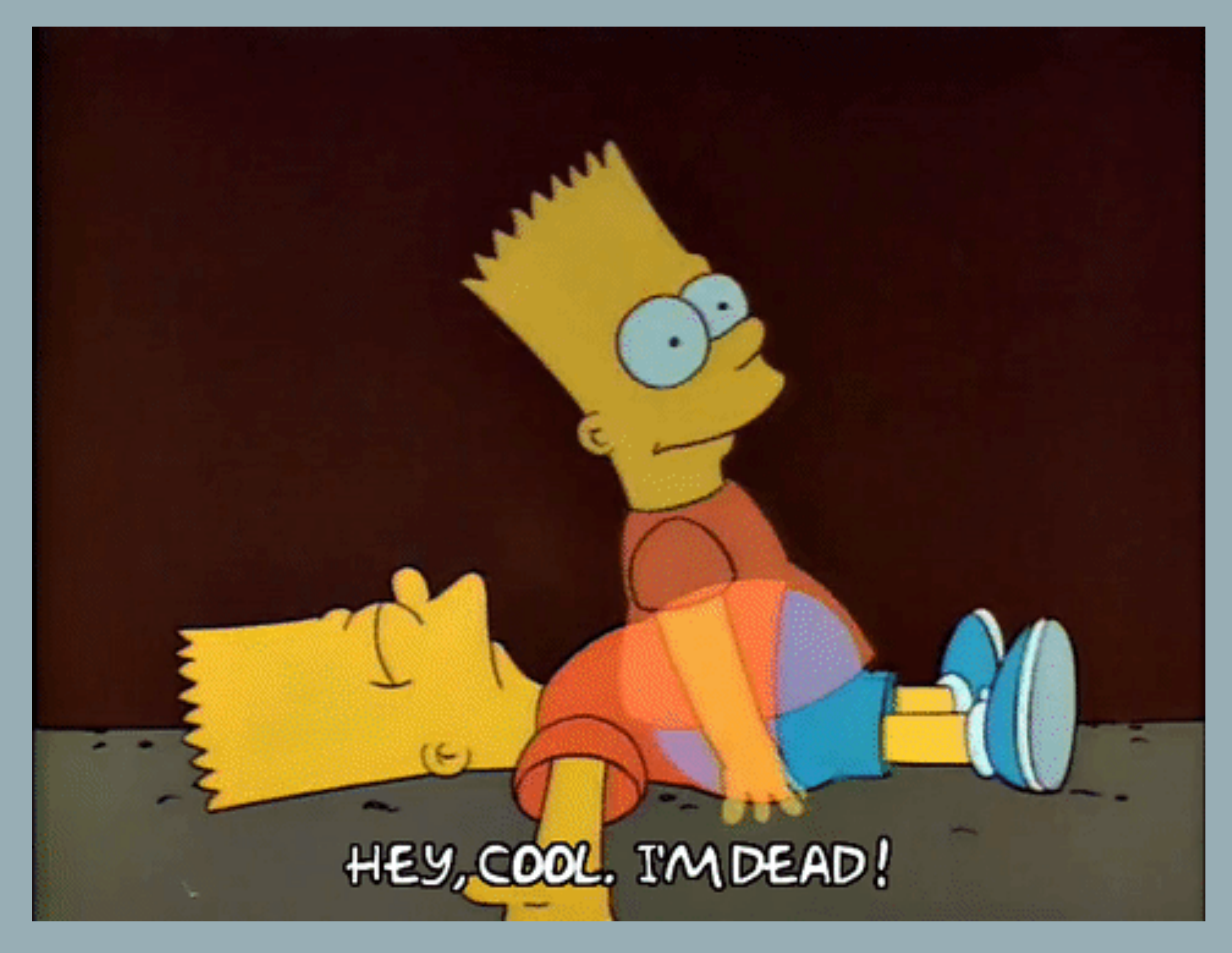
It allows us to preserve resources

☑ Pros

It helps us minimise waste products

It can reduce infectious disease rates

Cons



It creates an all-or-nothing approach

It is a field of research with many unknowns

☒ Cons

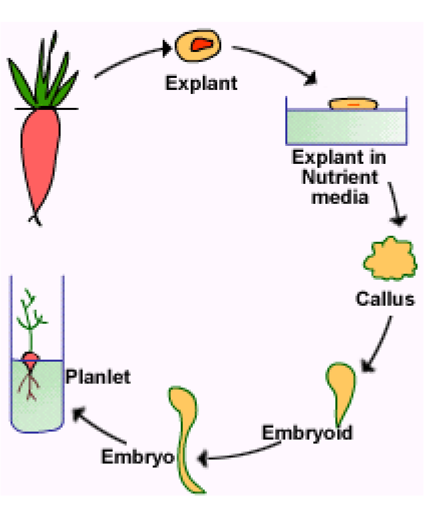
It can be used for destruction

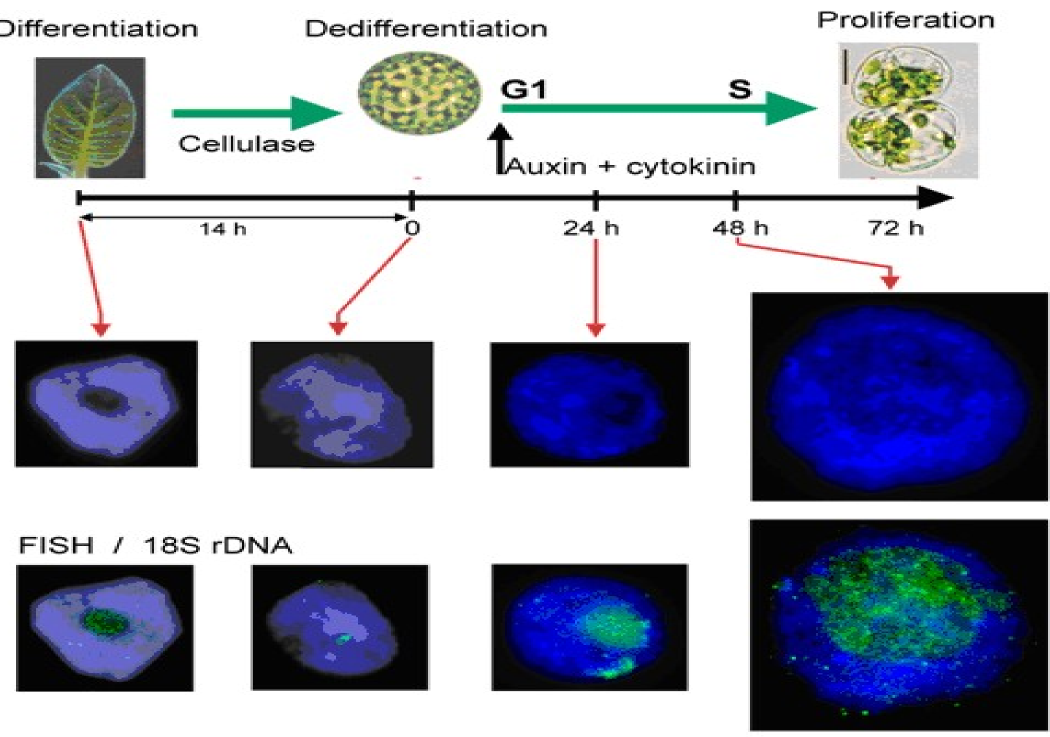
It could ruin croplands

Important principles

Totipotency

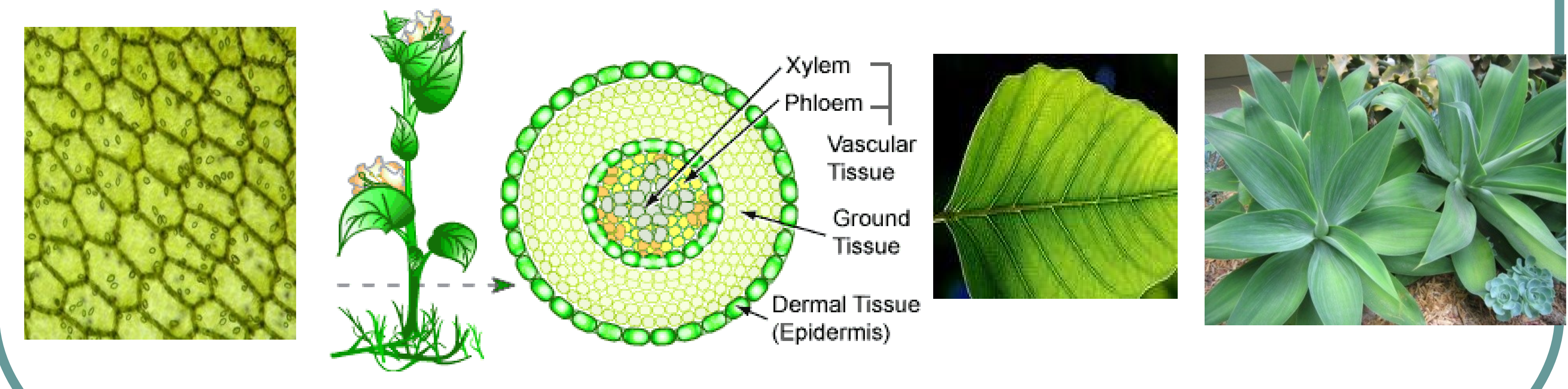
Dedifferentiation





What is tissue culture?

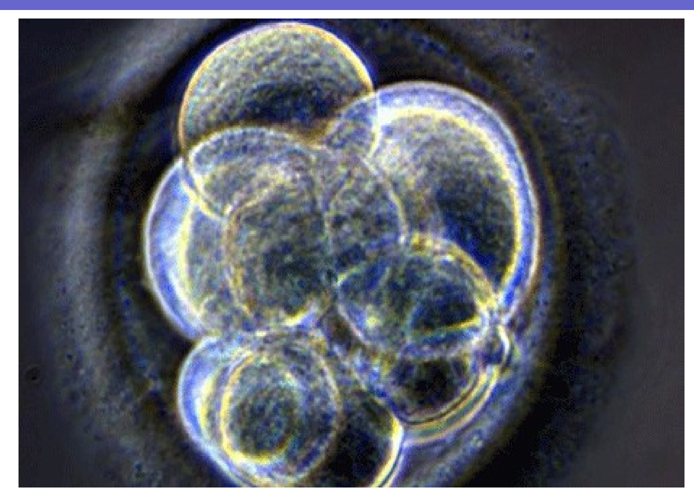
It is a technique of growing cells, tissues, organs or whole organism *in vitro* (in glass) on artificial culture medium under aseptic and controlled conditions.

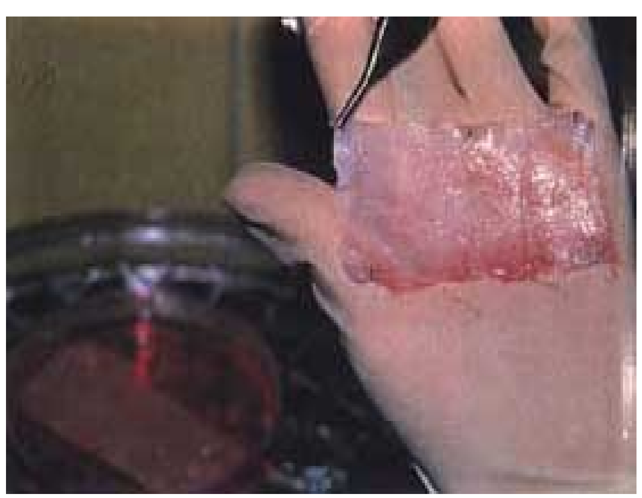


Types of tissue culture

- Plant tissue culture
- Animal tissue culture

Animal tissue culture





Plant tissue culture

Micropropagation

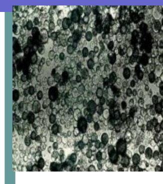
- Rapid vegetative propagation of several agricultural and horticultural crops.
- Replacing the conventional methods of propagation.

The mass multiplication of agricultural, horticultural, medicinal and other desirable plants by tissue culture techniques is known as micropropagation/clonal propagation.

Clone

Genetically same genome

History



H. Haberlandt (1902) attempted to culture isolated mesophyll cells but not succeeded.

R.J. Guatheret (1939) callus culture of carrot.

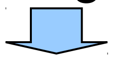
F. Skoog and C.O. Miller (1957) put forth the Hormone hypothesis

S.G. Guha and S.C. Maheshwari (1966) cultured pollens to obtain haploid plant.

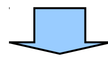
A.F. Mascarens (1991) induced flowering in bamboo plant by tissue culture technique.

Steps involved in the *in vitro* micropropagation

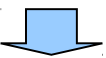
Cleaning of glassware



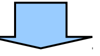
Preparation of nutrient medium



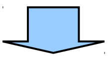
Selection and sterilization of *explant*.



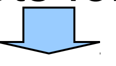
Inoculation of aseptic *explant* in to nutrient medium.



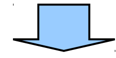
Proliferation of shoots on a multiplication medium.



Transfer of shoots for sub-culturing.



Rooting and hardening of plantlets



Field trials.

Cleaning of glassware

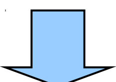
Borosilicate glassware (Corning/Pyrex) is used. Graduated measuring

cylinders,	conical flasks	beakers
petridishes,	pipettes (2 ml, 5 ml and 10 ml)	glass rods
centrifuge tubes	culture vials,	culture tubes

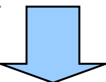
bottles

Procedure for cleaning of glassware

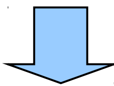
Soak glassware in 10% soap water (teepol) for 1 hour.



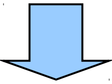
Transfer glassware to conc. HCl and keep for 2 hours.



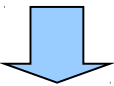
Rinse glassware in tap water.



Wash the glassware at least twice with distilled water.

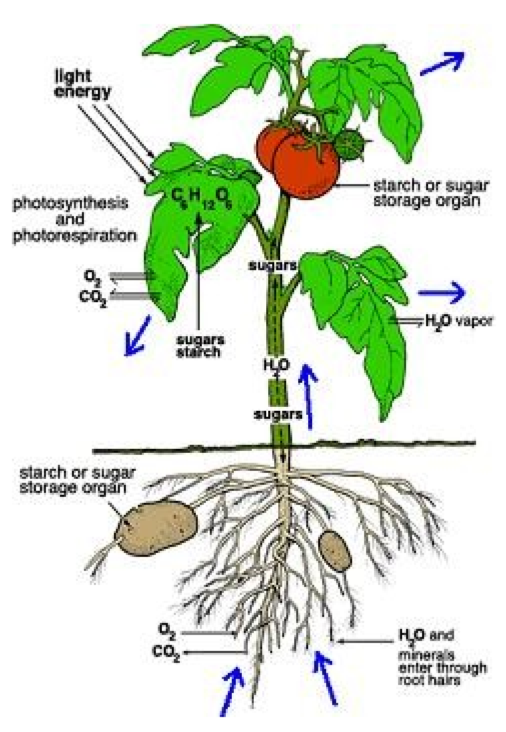


Keep glassware for drying in oven at 100 ^{0}C for 1 hour.

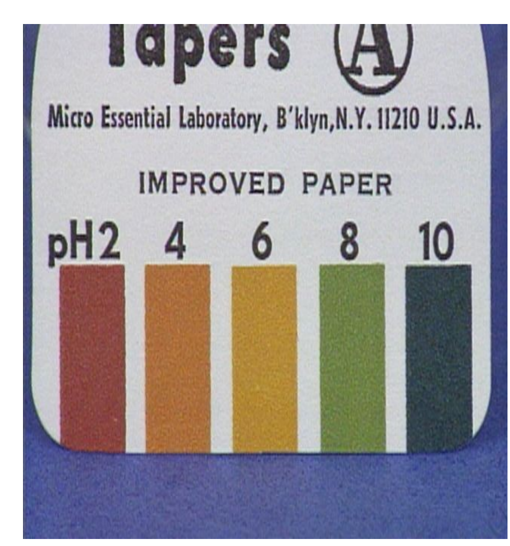


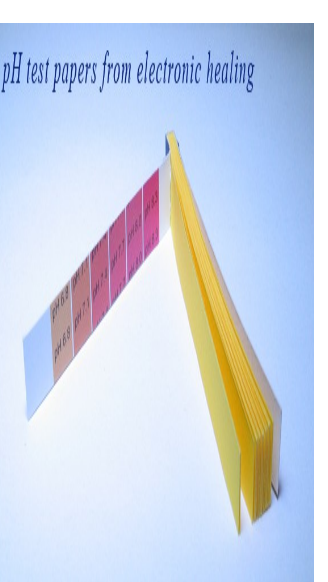
Autoclave/ keep glassware in oven at 140-160 ^{0}C for 2 hours.

Nutrient medium



Measuring of PH

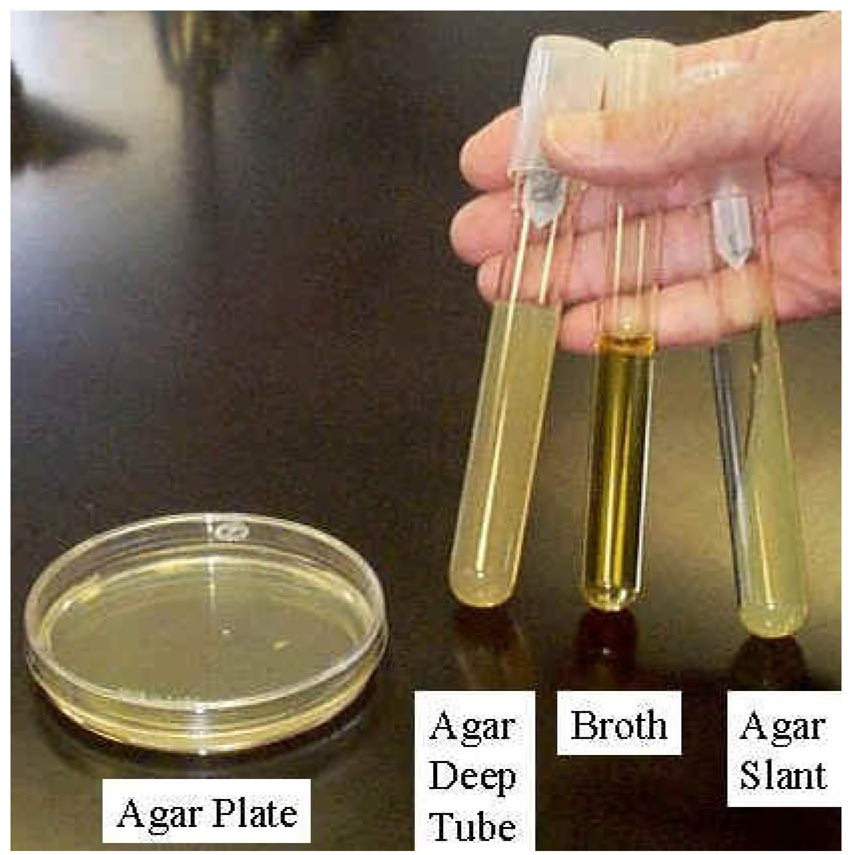




Autoclave for sterilization

VERTICAL AUTOCLAVE - FULLY AUTOMATIC

Medium preparation



Contd.

- Plant tissues/organs are grown *in vitro* on a suitable artificially prepared nutrient medium/culture medium.
- Single medium can not be used for the all types of plants and organs.
- Commonly used medium are Murashige and Skoog (MS), Nitsch, Gamborg, White, etc.
- MS medium is the most commonly used for plant tissue culture.
- Medium is composed of inorganic salts, iron, vitamins, amino acids, plant hormones and a carbohydrate supply.

Composition of nutrient medium

Table 1. Inorganic salt composition of Murashige and Skoog (13), Hoagland and Arnon (7) and White's (20) media.

Ingredients	Media		
	Murashige and Skoog	Hoagland and Arnon	White
Macronutrients (µmoles/liter)			
Nitrogen	60.0	15.0	2.0
NH_4^+	20.6	-	-
NO_3^-	39.4	15.0	2.0
Phosphorus	20.0	1.0	0.1
Potassium	1.3	6.0	1.7
Calcium	3.0	5.0	1.2
Magnesium	3.0	2.0	3.0
Sulfur	3.2	2.0	4.5
Micronutrients (µmoles/liter)			
Boron	100.0	46.3	-
Chlorine	2,993.0	10.9	870.0
Cobalt	0.1	-	-
Cooper	0.2	0.3	-
Iodine	5.0	-	4.5
Iron	10.0	9.0	10.0
Manganese	103.0	10.9	30.0
Molybdenum	1.1	0.1	-
Sodium	3.2	-	-
Zinc	3.0	0.8	9.0

- Salts are supplied in the form of macronutrients viz. N, Mg, K, Ca, P
- Micronutrients Cu, Ni, Mn, Co, etc.
- Iron is supplied in the chelated, Fe-EDTA (Ferric-Sodium Ethylene-Amine Tetra Acetate) form.
- Vitamins viz. meso-inositol, thiamin (B1), nicotinic acid (B3), pyridoxine (B6), etc.
- Aminoacids, mostly glycine is used.
- Carbohydrate is supplied usually in the form of sucrose.

- Phytohormones (auxins and cytokinins), their chemical form, concentration and ratio may vary from plant to plant.
- In general Auxins, such as IAA (Indole Acetic Acid) NAA (Naphthalene Acetic Acid), IBA (Indole Butyric acid); Cytokinins viz. Kinetin (6-furfuryl amino purine) 6-BAP (6, Benzyl Amino Purine) and Zeatin are used in nutrient medium.

Types of medium

Chemically defined nutrient medium

Chemically undefined nutrient medium:

Complex additives viz. coconut milk, Casein hydrolysate, yeast extract, water melon juice, etc. are added in the medium.

1. Solid medium: 6-8% agar-agar
2. Semi solid medium: Less amount of agar
3. Liquid medium: Agar is not added. It is used for cell suspension culture.

Preparation of stock solutions

- It is convenient to prepare stock solutions.
- When mixed together in appropriate quantities constitutes basal medium.
- It is not feasible to weigh and mix all the constituents of the nutrient medium for the preparation of the small quantity of the nutrient medium.
- It also provides flexibility to try different combinations of the nutrient medium.

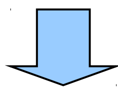
Sterilization

- Culture medium supports the growth of microbes e.g bacteria, fungi, etc. these grow fast and kills the plant cells.
- Microbes may come from glass vials, instruments, nutrient medium and also from the plant material.
- Therefore, the surface of plant tissue and all non-living articles including nutrient medium must be sterilized.
- **Sterilization of non-living articles:** The non-living articles viz. Nutrient medium, glassware, distilled water, instruments (wrapped with brown paper) are sterilized by autoclaving under steam at a 15 lb/inc2 and temperature 121^{0}C for 15 min. The glassware can also be sterilized by heating in oven at 150oC for 3-4 hrs. The thermoilabile compounds are sterilized by passing through the bacterial filters.

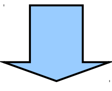
Sterilization of the plant material (Surface) sterilization

- The plant material should be surface sterilized to remove the surface borne micro-organisms.

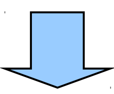
Water



10% v/v solution of liquid detergent (Teepol) for 10-15 min.



70% ethyl alcohol for 1 min. in front of laminar air flow.



Treatment with 0.1% HgCl2 (W/V) or 5-10% sodium hypochlorite.

Incubation of culture

- Cultures are incubated in a culture room where light, temperature and humidity are controlled.
- For some tissues dark is essential while for some both dark and light conditions are required.
- Humidity has also some effect.
- The cultures are incubated on culture rack at 25-28 oC constant temperature. Culture tubes are placed at 35-40o inclined position.
- Culture to give a light intensity of 4-10 X 103 lux for 16 hrs.

Subculturing

- Transfer of cell or tissue from old culture medium to fresh culture medium within definite time period.
- It provides sufficient space and nutrients to the growing plantlet.
- Multiplication of the callus.

Rooting

- It is the induction and development of adventitious roots on the proliferated shoots.
- Root formation is induced in a medium with high auxin and low cytokinins concentrations.
- Shoot tip or single node explant is used.
- Culture medium is maintained in a green house/mist chamber.
- Activated charcoal is frequently added to absorb root-inhibiting agents.

Hardening

- Healthy/elite plantlets are exposed to the natural conditions in a step wise manner.
- It is a gradual acclimatization of *in vitro* grown plants to *in vivo* condition.
- The plantlets are transferred to the pots/polyghene bag and immediately irrigated with inorganic/nutrient solution.
- Plants are kept in the hardening room where controlled conditions of light, humidity and temperature are maintained.
- Plants are maintained under high humidity for 10-20 days and subsequently transferred in the field so as to grow under natural conditions. The success rate of micropropagation depends on the survival of the plantlets when transferred from culture to the soil (field).

Laboratory setup

Space for washing and storage.

Sterilization room

Inoculation room

Culture room (incubation room)

Observation and inspection room.

Data collection and management room.

Tissue culture units

- Universities
- Research laboratories
- Private firms
- Nurseries

Application of tissue culture

- Rapid propagation.
- Minimum growing space is required.
- Multiplication of medicinal plants.
- Pathogen free plants -meristem culture.
- It is useful in the plants like papaya, coconut, etc.
- Large number of plants can be stored in the small space.

Contd.

- Problems with seed and vegetative propagation overcome.
- Artificial seeds - do not under go seed dormancy.
- Uniformity of characters.
- Seedless fruit propagated easily.
- *In vitro* cloning enables genetic manipulation,
- Hybrids with desired traits can be obtained by this method.
- Transgenic plants produced by tissue culture technique.
- Rare and endangered plants.
- Early flowering can be induced by tissue culture technique e.g. bamboo.

- There is potential danger of spreading of plants diseases through a diseased material in a large number of plants.
- It is not feasible for some tress, especially for some gymnosperms.
- In some cases multiple shooting takes place but rooting is difficult.
- Contamination in the culture room is a serious problem.
- In some cases shoots show decline in the rate of growth and plant die called vertrification.

Tissue Culture

Basic principles and terminology

Introduction

- What is plant tissue culture?
  - The growth or regeneration of plant cells, tissues, organs or whole plants in artificial medium under aseptic conditions.

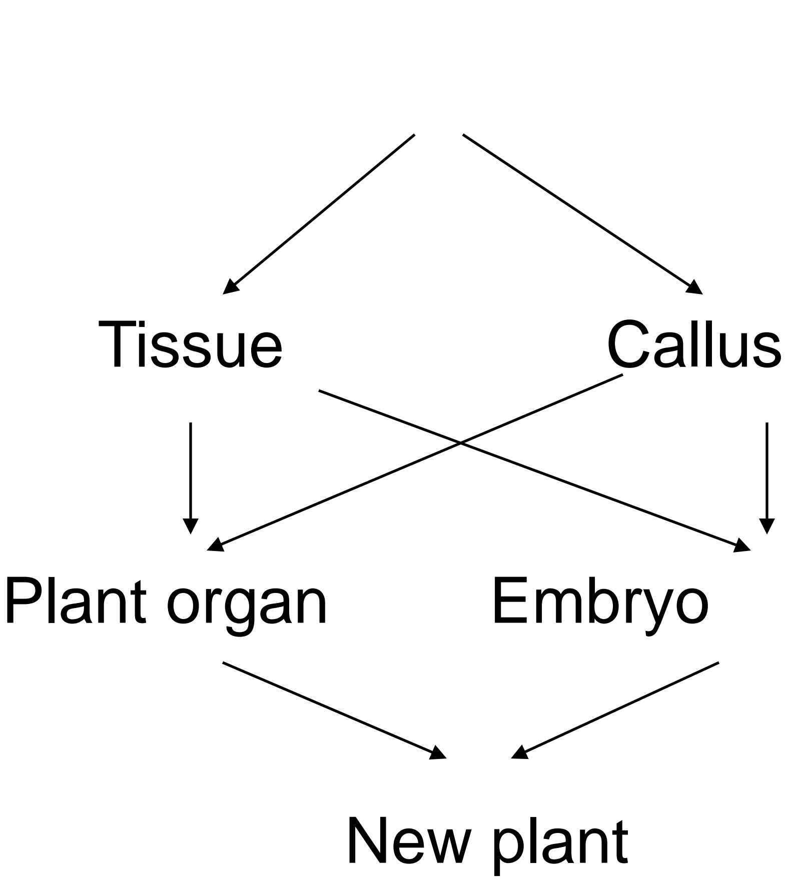
Introduction

- There are numerous methods to propagate plants in tissue culture.
- But the one principle that is constant is **totipotency** – all plants and plant parts have this potential.

Why does tissue culture work?

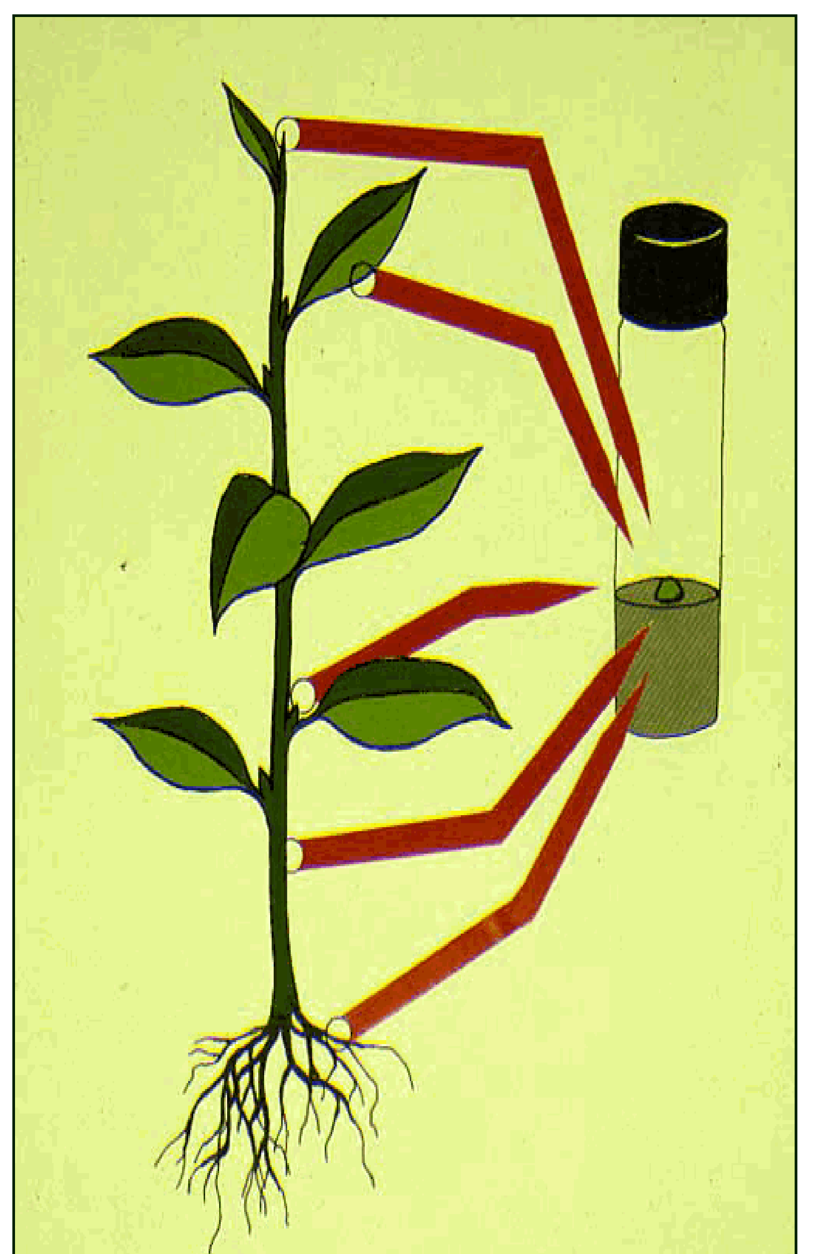
- Totipotency:
  - The ability of a cell to differentiate and develop into a whole plant when given the correct conditions. This is because every cell has the genetic potential of the parent plant.

Totipotency



Terminology

- Explant
  - Living tissue transferred from a plant to an artificial medium for culture.
  - It can be any portion of the shoot, leaves, roots, flower or cells from a plant.



Terminology

- *In vitro* culture
  - From Latin- “within the glass” performing an experiment in a test tube.
  - All types of culture including animal cells, *in vitro* fertilization, etc.

- Tissue culture
  - Inclusive term for growth of cells and tissues in a sterile environment
    - undifferentiated plant cells
    - plant callus
    - plant tissue

Terminology

- Micropropagation
  - The production of whole plants from small sections of a plant, called an “explant”.
    - Apical bud
    - Axillary bud
    - Meristem
  - Usually the method used by commercial tissue culture laboratories is micropropagation, since a whole plant (including shoots and roots) is produced, which is genetically identical to the mother plant.
  - Nowadays, tissue culture, *in vitro* culture and micropropagation are sometimes used interchangeably.

Terminology

- Motherplant
- *Ex vitro*
- *In vivo*
- Competent cell

Subculture

- After a period of time, it becomes necessary, due to nutrient depletion and medium drying, to transfer organs and tissues to fresh media.

- In general, callus cultures are subcultured every 4-6 weeks. Theoretically plant cell and tissue cultures may be maintained indefinitely by serial subculturing.

Introduction

- Type of cells
  - Meristematic cells
    - undifferentiated cells at shoot and root tips.
    - greatest potential to produce cells that will become shoots or roots.
  - Parenchyma cells
    - thin-walled cells that make up the bulk of most non-woody structures.
    - can be induced to divide and differentiate.

Types of tissue culture

1. Organized culture:

- The culture of whole or parts of a plant. The characteristics and organizational structure of a plant or organ is maintained.

- Axillary bud culture
- Terminal bud culture
- Seed culture
- Embryo culture
- Ovary culture
- Pollen culture

Types of tissue culture

2. Unorganized culture:
   - Callus culture
   - Cell suspension culture
   - Organogenesis
   - Somatic embryogenesis
   - Protoplast culture

Types of tissue culture

- The type of tissue culture techniques applied is dependent on the type of explant and what one wants to achieve.

- Techniques mentioned above is mainly used for propagation and multiplication.

- Other techniques include:
  - Micrografting – to eliminate viruses, to root microcuttings.
  - *In vitro* pollination
  - *In vitro* fertilization

  } Used by plant breeders to create new varieties

Advantages

- Mass production of various plant cultivars
  - 6 million plants per year from one explant.
  - Much higher production rate than other asexual propagation methods.
- Especially beneficial for:
  - Plants in high demand or valuable plants.
  - Plants that are slow or difficult to propagate.
  - Endangered species.

Advantages

- Production of pathogen-free plants
  - Maintaining disease-free plants by micropropagation.

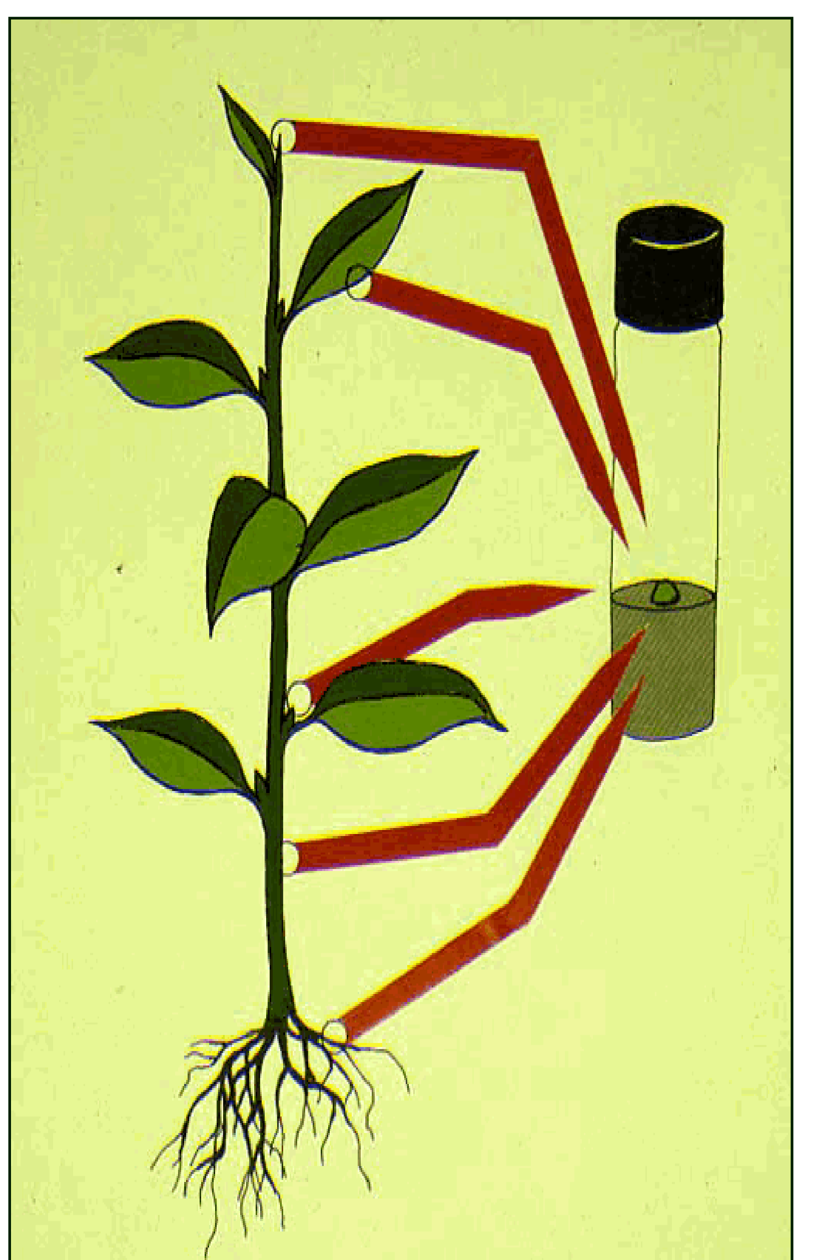
- Germplasm preservation
  - Germplasm: the DNA of a species
  - In the past: seeds
    - limited shelf-life
    - don’t preserve uniform characteristic (variability)

Advantages

- Continuous year round production
  - Unaffected by climate
- Propagated in controlled lab conditions
  - The ability to change specific conditions to meet the needs of a particular plant species.
  - Mainly, nutrient, light and temperature requirements.

Advantages

- The original plant is not destroyed in the process - a factor of considerable importance to the owner of a rare or unusual plant.



Disadvantages

- Specialized equipment required
  - Laminar flow cabinets
  - Autoclave
  - Water purification systems
  - Glassware etc…
- High labor cost is the most limiting factor
  - Skilled labor required

Disadvantages

- Contamination risks
  - Maintenance of aseptic (sterile) environment difficult.
  - Rapid spread of contaminants = widespread loss.
- Risk of mutation arising
  - Artificial environment induces mutations.
- Responses to tissue culture conditions varies
  - Trial and error to determine optimum media or conditions ◦

Factors affecting tissue culture

- The areas in which tissue culture techniques can be used are very wide.

- The choice of technique is dependent on what one wants to achieve. It may be **mass production**, **breeding of new varieties**, or producing **virus-free plants**.

- To be able to successfully propagate plants *in vitro*, understanding **how** and **why** these factors affect plant growth in an *in vitro* environment is crucial.

Factors affecting tissue culture

- The *in vitro* growth and development of a plant is determined by a number of factors:
  - The genetic make-up of the plant
  - Source of explants
  - Nutrients
  - Environmental factors: light, temperature, pH, O_2 and CO_2 concentrations.

Factors affecting tissue culture

- The genetic make-up of the plant.
  - The genetic make-up is a decisive factor at every stage in the plant.
  - It determines, for example, if a plant is a monocotyledon or dicotyledon, or which temperature is optimal for growth.
  - The type of *in vitro* environment that must be created in the lab to ensure that growth and development of the explant takes place, is totally dependent on the genotype of the plant.

Factors affecting tissue culture

- Source of explant
  - Young explant vs. old explant
  - Usually the younger, less differentiated explant, the better for tissue culture
  - Type of explant – leaf, stem, root, meristem, etc.

Factors affecting tissue culture

- Growth medium (Artificial)
  - Nutrients
  - Plant hormone
  - Vitamins

- Environmental factors (Controlled)
  - Light intensity
  - Photoperiod
  - Temperature
  - **Sterility**

Tissue Culture Applications

- ✓Micropropagation
- ✓Germplasm preservation
- ✓Somaclonal variation
- ✓dihaploid production
- ✓Protoplast fusion
- ✓Secondary metabolites production
- ✓Genetic engineering

Contents

1 Micro propagation

2 Somaclonal variation

3 virus free plants

4 synthetic seeds

5 Mutant selection

Production Of Viruses Free Plant

- ✓ Heat treatment.
- ✓ Meristemming.
- ✓ Not all cells in the plant are infected.

Production Of Viruses Free Plant

- ✓ Heat treatment.

  Plants grow faster than viruses at high temperatures.

- ✓ Meristemming.

  Viruses are transported from cell to cell through plasmodesmata and through the vascular tissue. Apical meristem often free of viruses. Trade off between infection and survival.

- ✓ Not all cells in the plant are infected.

  Adventitious shoots formed from single cells can give virus-free shoots.

Production Of Viruses Free Plant

	Conventional	Micropropagation
Duration:	6 years	2 years
Labor:	Dig & replant every 2 years; unskilled (Inexpensive)	Subculture every 4 weeks; skilled (more expensive)
Space:	More, but less expensive (field)	Less, but more expensive (laboratory)
Required to prevent viral infection:	Screening, fumigation, spraying	None

Mutation

- May involve any trait
- All kind of transition are encountered, from drastic morphological changes deviations in physiology so minute as to be almost indiscernible
- Harmful or even lethal

Mutant selection

- May involve any trait
- All kind of transition are encountered, from drastic morphological changes deviations in physiology so minute as to be almost indiscernible
- Harmful or even lethal

Mutation Breeding

- **Advantages**
  - Screen very high populations (cell based) Can apply selection to single cells
- **Disadvantages**
  - Many mutations are non-heritable
  - Requires dominant mutation (or double recessive mutation); most mutations are recessive

Type of mutation

- Spontaneous (natural) mutation
  1. Some have played an outstanding role in development of valuable crop cultivars and hybrids
  2. Unfortunately, it can not form the basis of modern plant breeding due to its low frequency and difficulties in detection
- Induced mutation

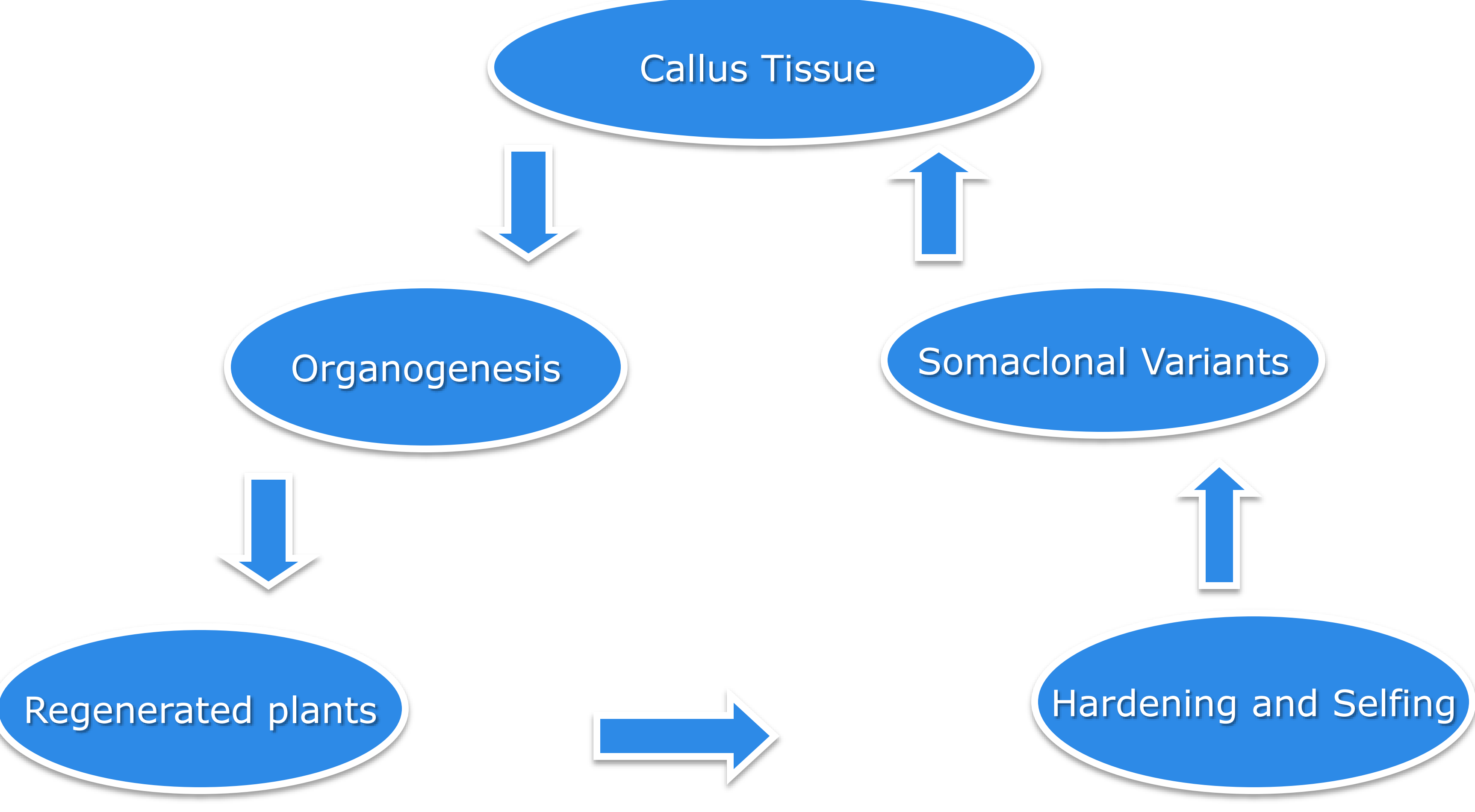
Somaclonal variation

Somaclonal variation

1. **Genetic (Heritable Variations)**
    - Pre-existing variations in the somatic cells of explant
    - Caused by mutations and other DNA changes
    - Occur at high frequency

2. **Epigenetic (Non-heritable Variations)**
    - Variations generated during tissue culture
    - Caused by temporary phenotypic changes
    - Occur at low frequency

Somaclonal variation



Somaclonal variation

Causes of Somaclonal Variations

Physiological Cause

Genetic Cause

Biochemical Cause

Advantages of Somaclonal Variations

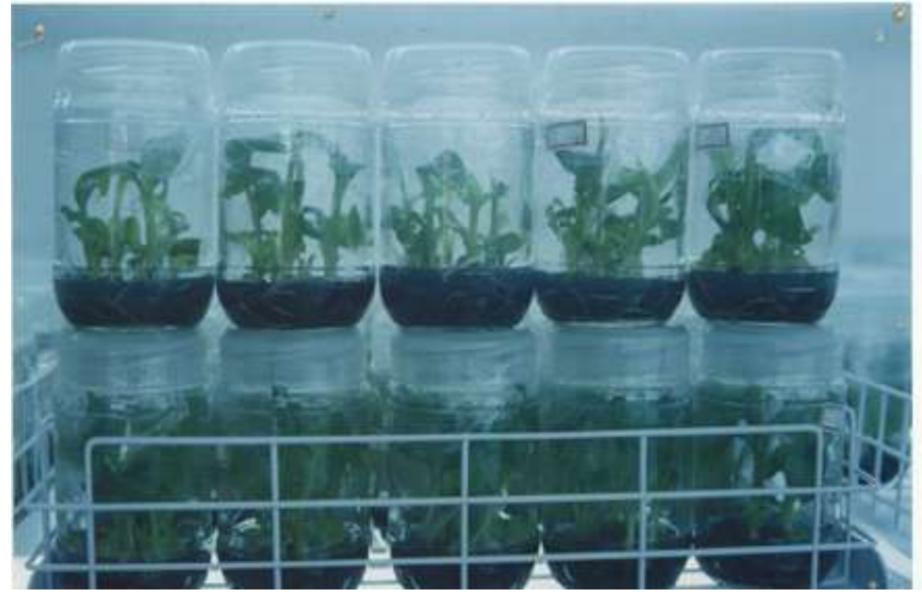
- Help in crop improvement
- Creation of additional genetic varitions
- Increased and improved production of secondary metabolites
- Selection of plants resistant to various toxins, herbicides, high salt concentration and mineral toxicity
- Suitable for breeding of tree species

MicroPropagtion

Stage 1 – Selection & preparation of the mother plant

Stage 2 - Initiation of culture

Stage 4 – Rooting



Stage 5 - Transfer to soil

Micro propagation

1. The main advantage of micropropagation is the production of many plants that are clones of each other.

2. Micropropagation can be used to produce disease-free plants.

3. Micropropagation produces rooted plantlets ready for growth, saving time for the grower when seeds or cuttings are slow to establish or grow.

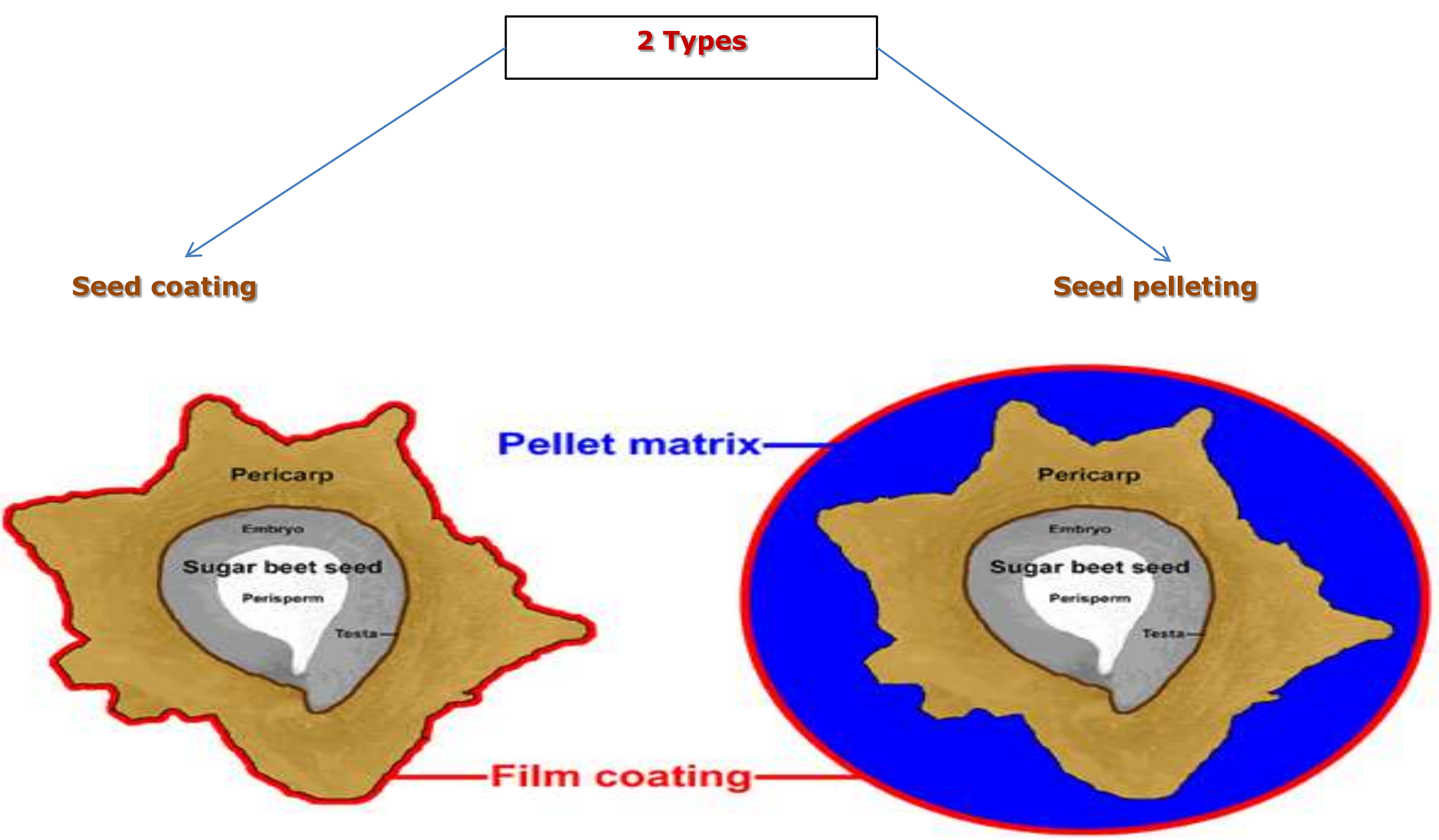
4. A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

Advantages

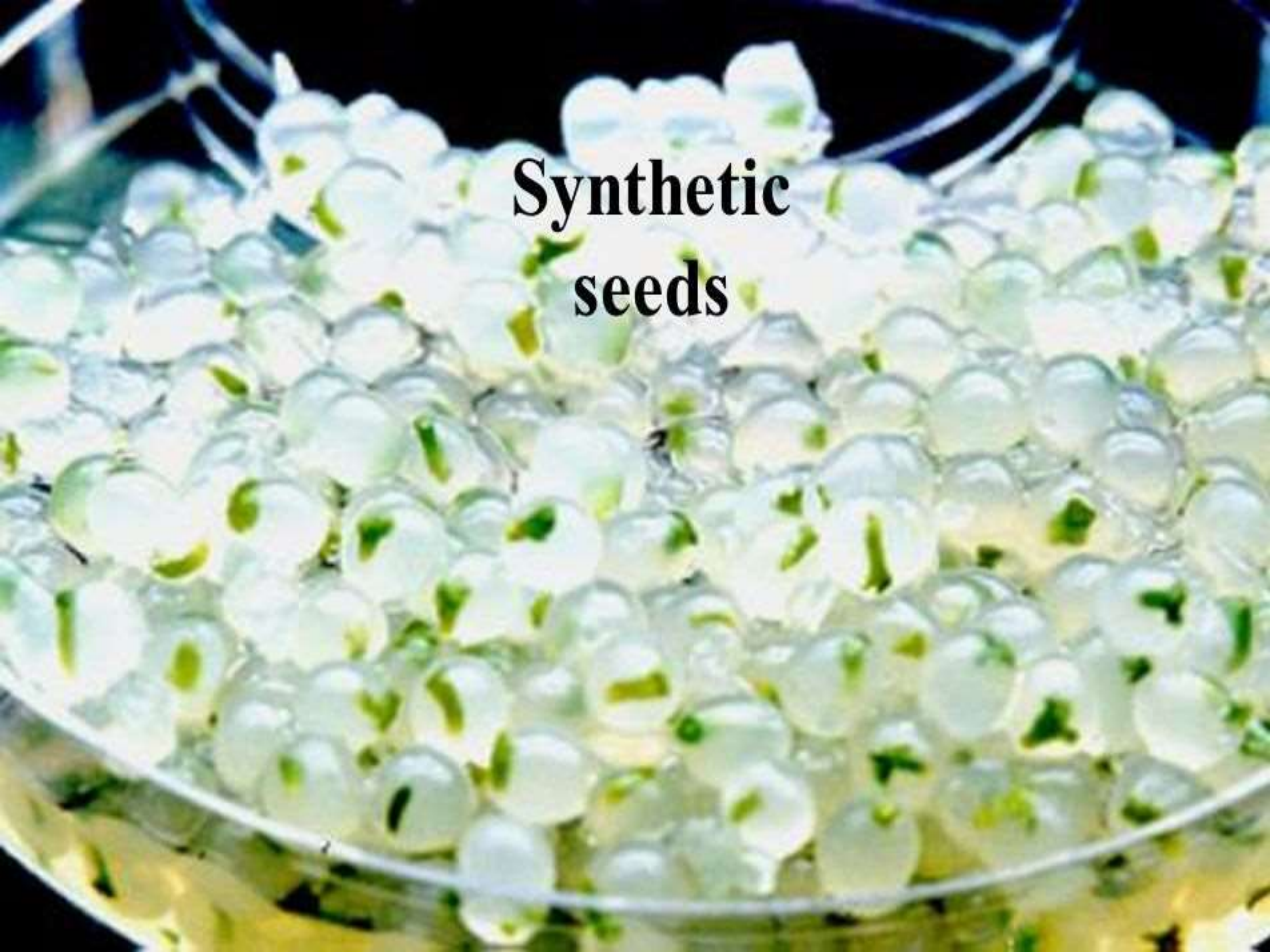
- Endangered species can be propagated using synthetic seed technology.
- Synthetic seeds can be directly used in fields
- Cereals, fruits and medicinal plants can be studied anywhere in the world using synthetic seeds.
- Synthetic seeds are small therefore they are easy to handle.

Production of synthetic seeds

Synthetic seed can be defined as the artificial encapsulation of somatic embryo, shoot buds or aggregates of cell or any tissues which has the ability to form a plant in in-vitro



Culture Technique	Applications
Meristem Culture	Virus free plant production
Seed Culture	Increase efficiency of seed germination
Callus Culture	Produce somatic embryo
Embryo Culture	Embryo rescue, Immature seed, Seed sterility
Anther Culture	Haploid Plant Production
Suspension Culture	Single Cell Production
Protoplast Culture	Somatic Hybrid Production


Synthetic
seeds

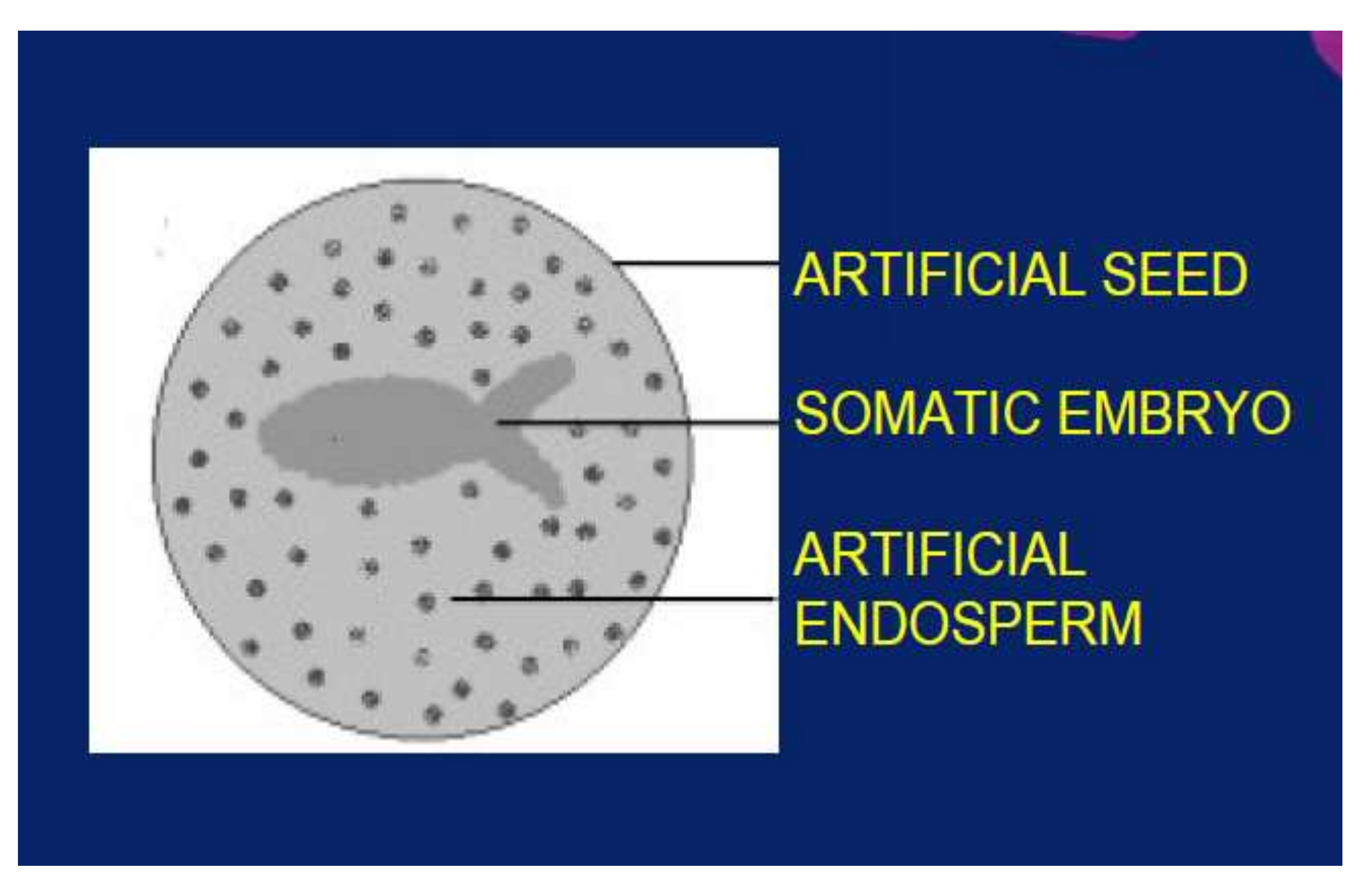
WHAT IS ARTIFICIAL SEED..?

- Artificial seed can be defined as artificial encapsulation of somatic embryos, shoot bud or aggregates of cell of any tissues which has the ability to form a plant in in-vitro or ex-vivo condition.
- Artificial seed have also been often referred to as synthetic seed.

HISTORY

- Artificial seeds were first introduced in **1970's** as a novel analogue to the plant seeds.
- The production of artificial seeds is useful for plants which do not produce viable seeds. It represents a method to propagate these plants.
- Artificial seeds are small sized and these provides further advantages in storage, handling and shipping.
- The term, "EMBLING" is used for the plants originated from synthetic seed.
- The use of synthetic varieties for commercial cultivation was first suggested in Maize (Hays & Garber, 1919).

The Concept of artificial seed



BASED ON THE TECHNIQUES TWO TYPES OF ARTIFICIAL SEEDS ARE PRODUCED

1. DESICCATED SYNTHETIC SEEDS- Desiccated synthetic seeds are produced nacked or polyoxyethylene glycol encapsulated somatic embryos. This type of synthetic seeds is produced in desiccation tolerant species plant.
2. HYDRATED SYNTHETIC SEEDS- Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogels like sodium alginate, potassium alginate, carrageenan, sodium pectate or sodium alginate with gelatine.

NEED FOR ARTIFICIAL PRODUCTION TECHNOLOGY

- Development of micro propagation technique will ensure abundant supply of desired plant species.
- Development of artificial seed production technology is currently considered as an effective and efficient method of propagation in several commercially important agronomic and horticultural crops.
- These artificial seed would also be a channel for new plant lines produced through biotechnological advances to be delivered directly to greenhouse and field.
- High volume propagation potential of somatic embryos combined with formation of synthetic seeds for low-cost delivery would open new vistas for clonal propagation in several commercially important crop species.

BASIC REQUIREMENT FOR THE PRODUCTION OF ARTIFICIAL SEEDS.

- One pre-requisite for the application of synthetic seed technology in micropropagation is the production of high quality,

1. Vigorous Somatic Embryos that can produce plants with frequencies comparable to natural seeds.
2. Inexpensive production of large numbers of high quality somatic embryos with synchronous maturation.
3. Encapsulation and coating systems, though important for delivery of somatic embryos, are not the limiting factors for the development of synthetic seeds.
4. Commercialization of synthetic seeds.

PROCEDURE FOR PRODUCTION OF ARTIFICIAL SEEDS

Establish somatic embryogenesis

↓

Mature somatic embryogenesis

↓

Synchronize and singulate somatic embryos

↓

Mass production of somatic embryos

↓

Standardization of encapsulation

↓

Standardization of artificial endosperm

↓

Mass production of artificial seeds

↓

Greenhouse and field planting

Methods for artificial seed encapsulation

- **Dropping method**
- Somatic embryos are dipped in hydrogel, this step encapsulate SEs.
- Hydrogel used may be any of the following.
- Alginate – sodium alginate, agar from see weeds, seed gums like guar gum, locust bean gum.
- Sodium alginate solution (1 – 5%), prepared in MS basal medium solution.
- SEs are dipped in this solution.
- These coated beads are added one by one into a complexation solution flask kept on magnetic stirrer and kept such for around 20-30 minutes.

Contii.

- Embryos get covered by calcium alginate which is a stable complex due to ionic bond formation, become harder, Seeds become harder.
- Then gelled embryos are washed with water or MS basal medium.
- The synthetic seeds are ready.

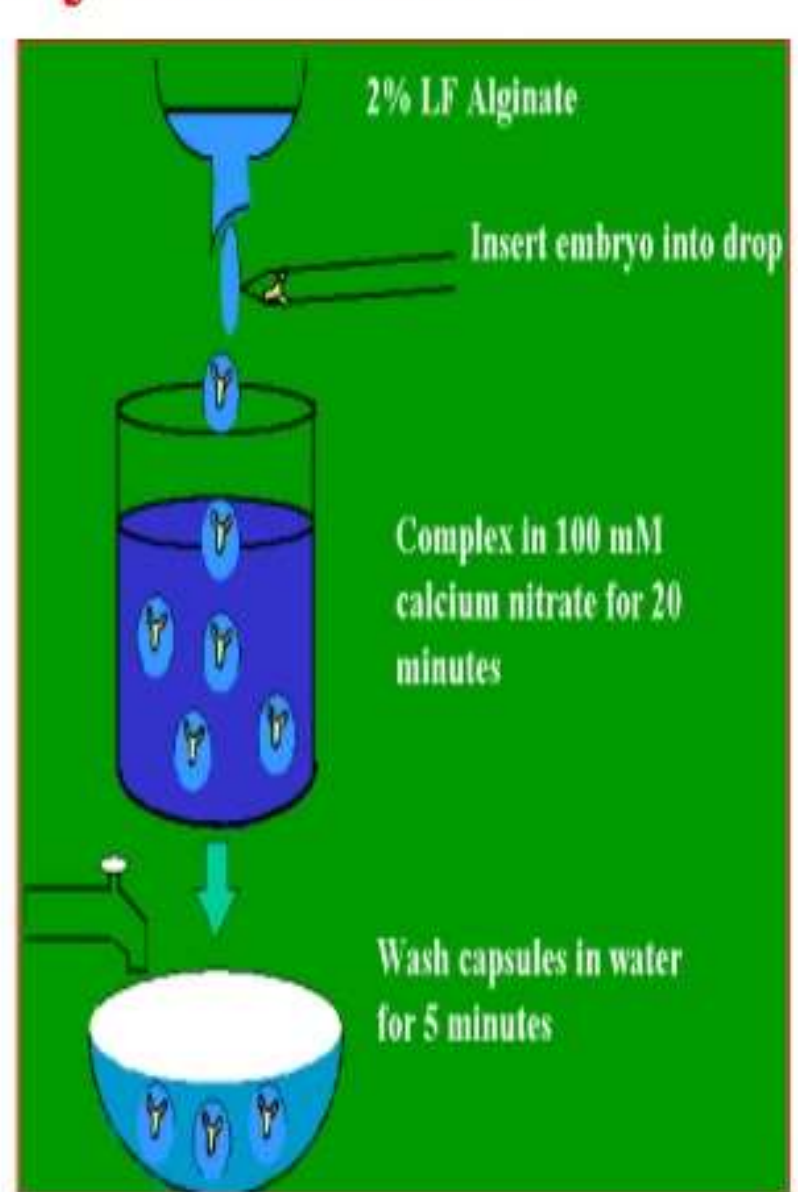
Encapsulation methods for synthetic seed

A) Dropping procedure

1) The most useful encapsulation system. Drip 2-3 % sodium alginate drops from at the tip of the funnel and the somatic embryos are inserted

2) Keep the encapsulated embryos complex in calcium salt for 20 min

3) Rinsed the capsules in water and then stored in a air tight container



Molding method

- This method follows simple procedure of mixing of embryos with temperature dependent gel (e.g. gel rite, agar).
- Cells get coated with the gel at lowering of the temperature.

ARTIFICIAL ENDOSPERM

- Somatic embryos lack seed coat (testa) and endosperm that provide protection and nutrition for zygotic embryos in developing seeds.
- To augment these deficiencies, addition of nutrients and growth regulators to the encapsulation matrix is desired, which serves as an artificial endosperm.
- These addition results in increase efficiency of germination and viability of encapsulated somatic embryos.
- These synthetic seeds can be stored for a longer period of time even upto 6 months without losing viabilty, especially when stored at 40°c.

ADDITION OF ADJUVANTS TO THE MATRIX

- To prevent the embryo from desiccation (state of extreme dryness) and mechanical injury, a number of useful materials such as nutrients, fungicides, pesticides, antibiotics and microorganisms (eg. rhizobia) may be incorporated into the encapsulation matrix.
- Incorporation of activated charcoal improves the conversion and vigour of the encapsulated somatic embryos and retains nutrients within the hydrogel capsule and slowly releases them to the growing embryo.

POTENTIAL USES OF ARTIFICIAL SEEDS

- Reduced costs of transplants(Cost effective)
- Direct greenhouse and field delivery of:
  - Elite, Select Genotypes
  - Large-scale mono cultures.
  - Carriers for adjuvant such as microorganisms, plant growth regulators, pesticides, fungicides, nutrients and antibiotics.

- Can be conceivably handled as seed using conventional planting equipment.
- it can be produced throughout the year.
- Conservation of germplasm
- Large production of identical embryos in short period of time.