# Biotechnology

# Cell culture and Hybridoma Technology

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# BIOTECHNOLOGY

- Biotechnology represents the integrated use of biochemistry, microbiology and engineering sciences for the manufacturing of various useful substances
- The term was introduced by **Karl Ereky** in 1917
- It began with the discovery of fermentation and developed through new techniques like cell culture, cell fusion, protoplast fusion, genetic engineering, recombinant DNA manipulations, Monoclonal antibody preparation, protein engineering etc.

- **Fermentation** : Denotes the chemical transformation of organic compounds by microbial metabolism in an anaerobic condition

- Glycerol -----using Yeast
- Acetone butanol ----- using *Clostridium acetobutylicum*
- Citric acid ----- using *Aspergillus niger*
- Pencillin ----- using *Pencillium notatum*

# Branches of Biotechnology

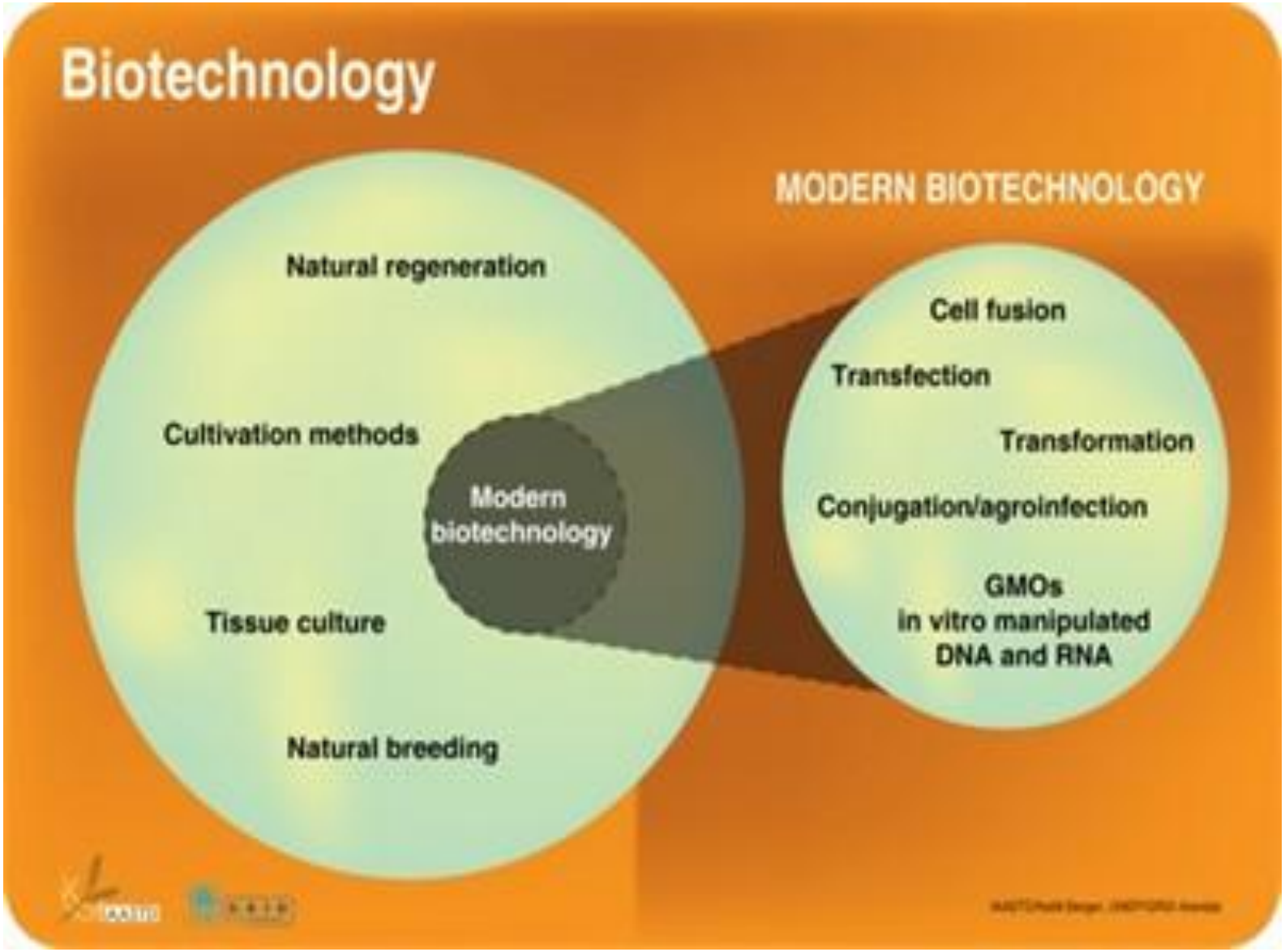
- **Medical/Red Biotechnology** – Recombinant vaccine, monoclonal antibodies, valuable drugs, gene therapy, DNA finger printing etc.
- **Indusrial/White Biotechnology** – Alcohol, antibiotics, enzymes, protein/enzyme engineering, microbial mining etc
- **Environmental Biotechnology** – Bioremediation, Biosensors, Bioleaching, Biofiltration etc.
- **Animal Biotechnology** – Transgenic animals for incresed milk, growth rate, disease resistance, production of valuable proteins in milk,urine, blood

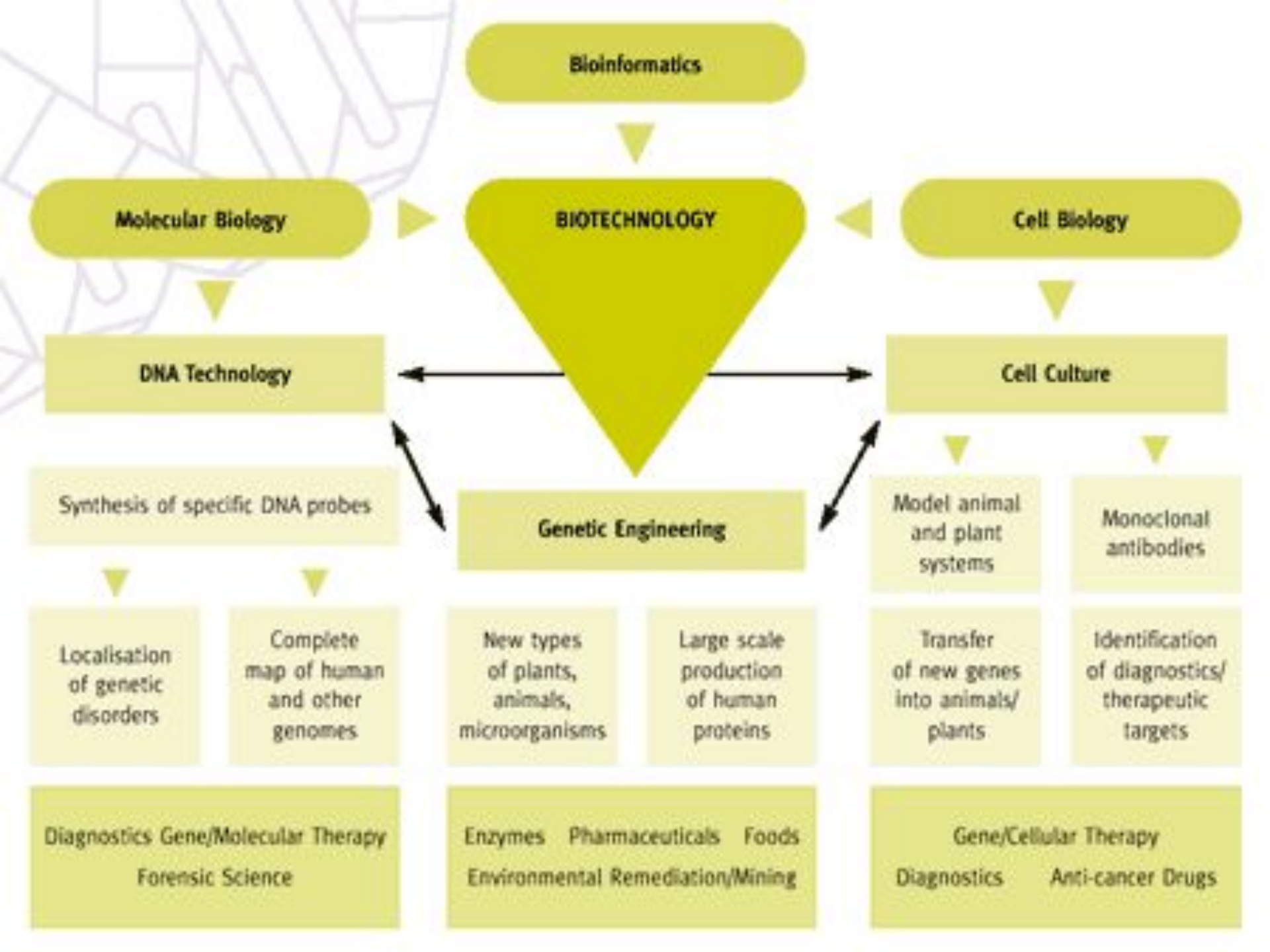
- **Plant Biotechnology** – Production of transgenic plants resistant to the attack of insects, viruses, herbicides etc.
- **Blue Biotechnology** – Deals with marine and aquatic applications

# Institutes

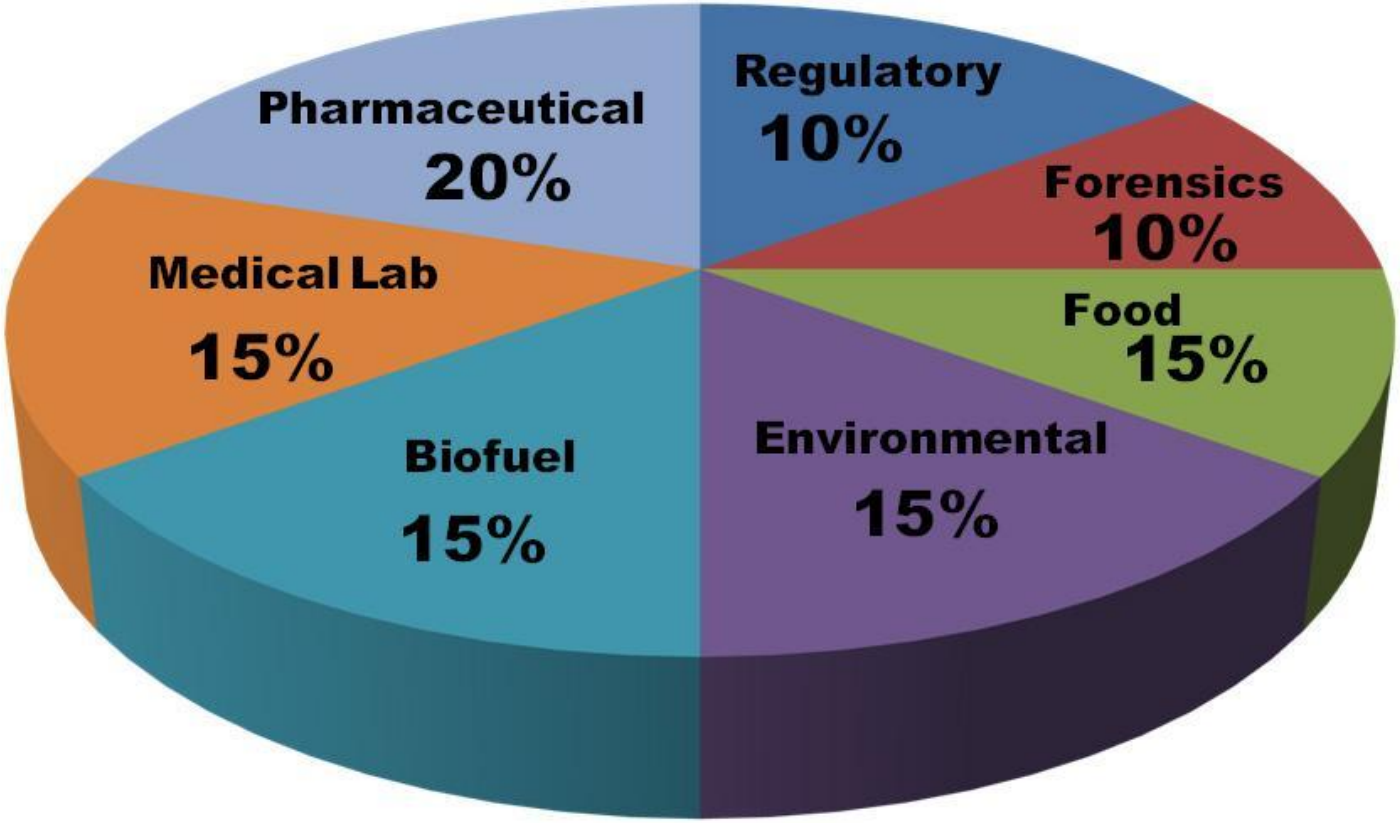
- NBTB – National Biotechnology Board
- DBT – Department of Biotechnology
- IARI – Indian Agricultural Research Institute, New Delhi [Bahadur Shastri Centre for Advance Research in Biotechnology]
- IVRI – Indian Veterinary Research Institute
- ICGEB – International Centre for Genetic Engineering and Biotechnology
- NDRI – National Diary Insttute

- ICAR – Indian Council for Agricultural Research
- TBGRI – Tropical Botanical Garden and Research Institute, Trivandrum
- IMTECH – Institute of Microbial technology Chandigarh


Biotechnology
MODERN BIOTECHNOLOGY
Natural regeneration
Cultivation methods
Modern biotechnology
Tissue culture
Natural breeding
Cell fusion
Transfection
Transformation
Conjugation/agroinfection
GMOs
in vitro manipulated
DNA and RNA


Bioinformatics
Molecular Biology
BIOTECHNOLOGY
Cell Biology
DNA Technology
Cell Culture
Synthesis of specific DNA probes
Genetic Engineering
Model animal and plant systems
Monoclonal antibodies
Localisation of genetic disorders
Complete map of human and other genomes
New types of plants, animals, microorganisms
Large scale production of human proteins
Transfer of new genes into animals/ plants
Identification of diagnostics/ therapeutic targets
Diagnostics Gene/Molecular Therapy
Forensic Science
Enzymes Pharmaceuticals Foods
Environmental Remediation/Mining
Gene/Cellular Therapy
Diagnostics Anti-cancer Drugs

# Biotechnology Curriculum by Industry



# CELL CUTURE

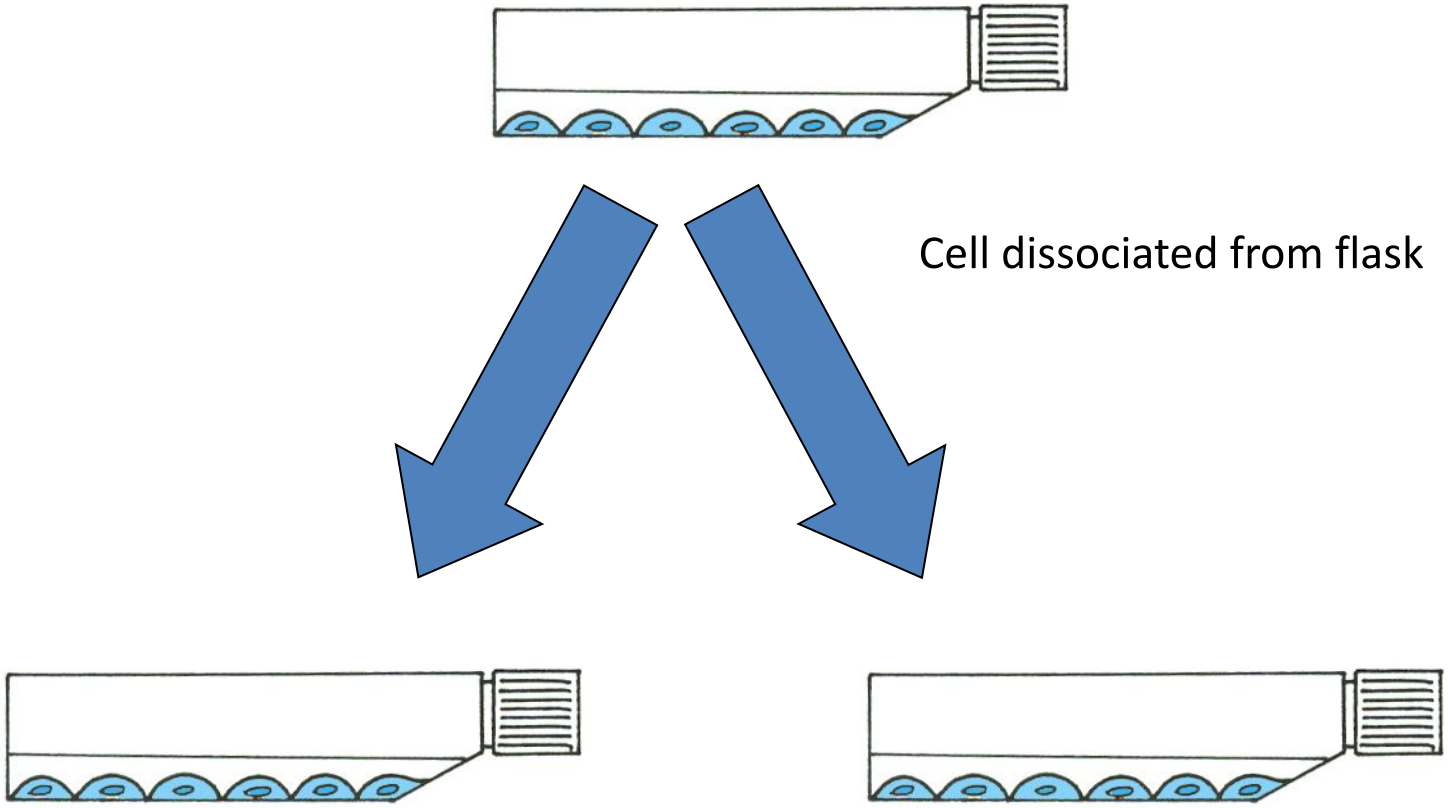
- **Cell culture** – is the complex process by which cells are grown under controlled conditions on a suitable nutrient medium

- Foundation was laid by **Jolly, Harrison and Carrel**

**A) Primary culture –** cell cultures prepared from directly from freshly isolated tissue source either by allowing cells to migrate passively or by disaggregating the tissue

- Mechanically
  - Mincing, shearing, sieves
- Enzymatically (proteases)
  - Trypsin, pronase, collagenase, dispase
- Chemically treating with chelating agents like EDTA

# Passaging or sub-culture

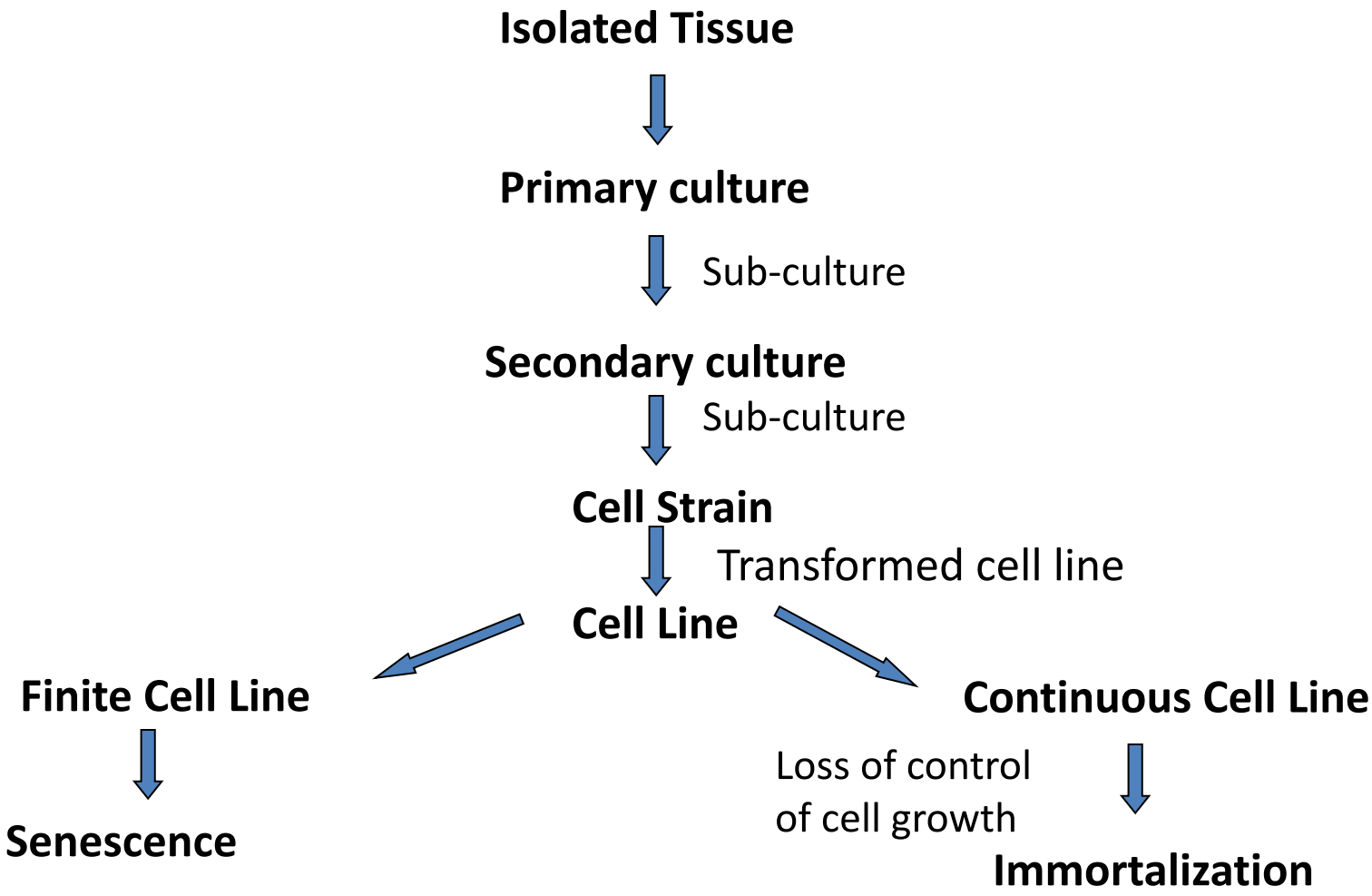


Cell dissociated from flask

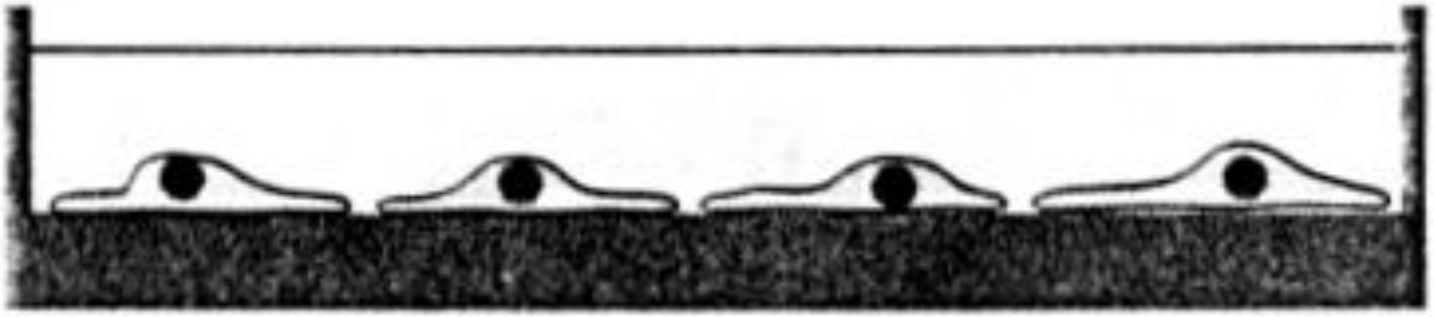
**Secondary culture**

- **SECONDARY CULTURAL -** Culture prepared by subculturing cells from a primary culture
- Extended culture – multipassage culture : Cell strain – A particular cell lineage having specific properties different from rest of the cells in the culture
- Established culture — with transformed cells : Cell line – Is a permanently established cell culture that will proliferate indefinitely if appropriate medium and space is provided

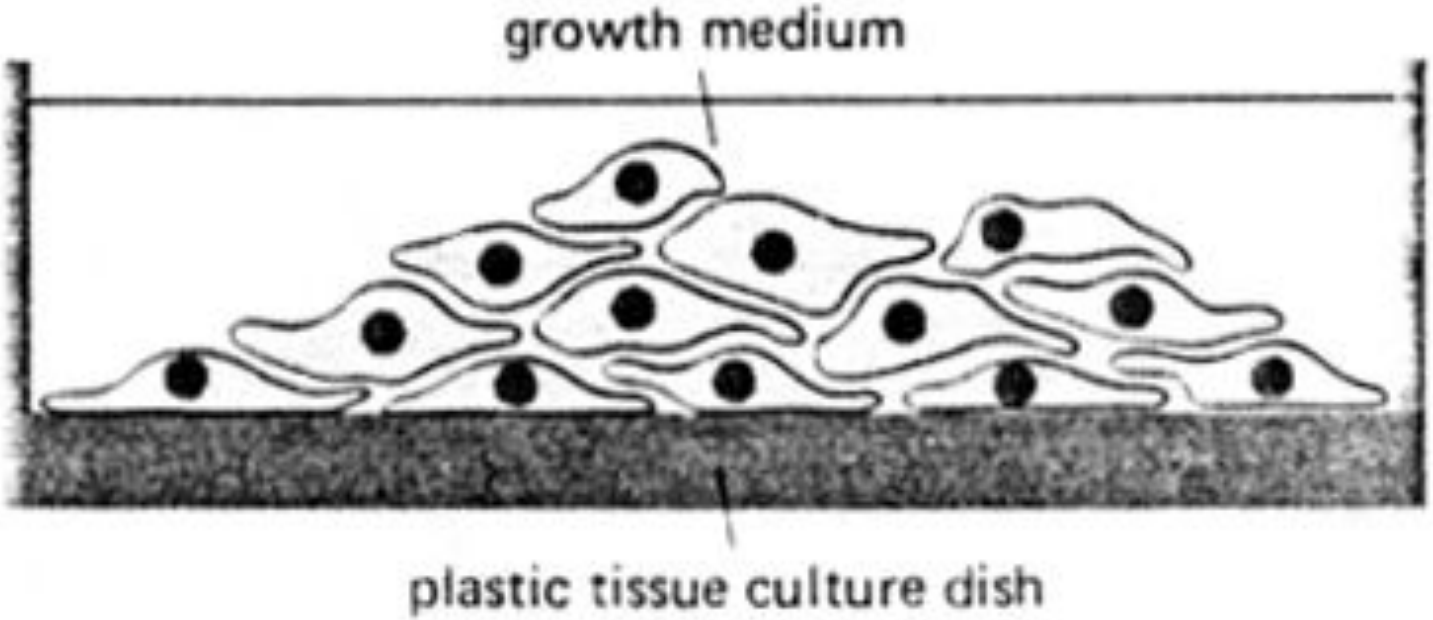
1. Finite cell line: dies after several sub-cultures
2. Continuous cell line: transformed ‘immortal’


Isolated Tissue
Primary culture
Sub-culture
Secondary culture
Sub-culture
Cell Strain
Transformed cell line
Cell Line
Finite Cell Line
Senescence
Continuous Cell Line
Loss of control
of cell growth
Immortalization

Contact inhibition & anchorage dependency– Finite cell line



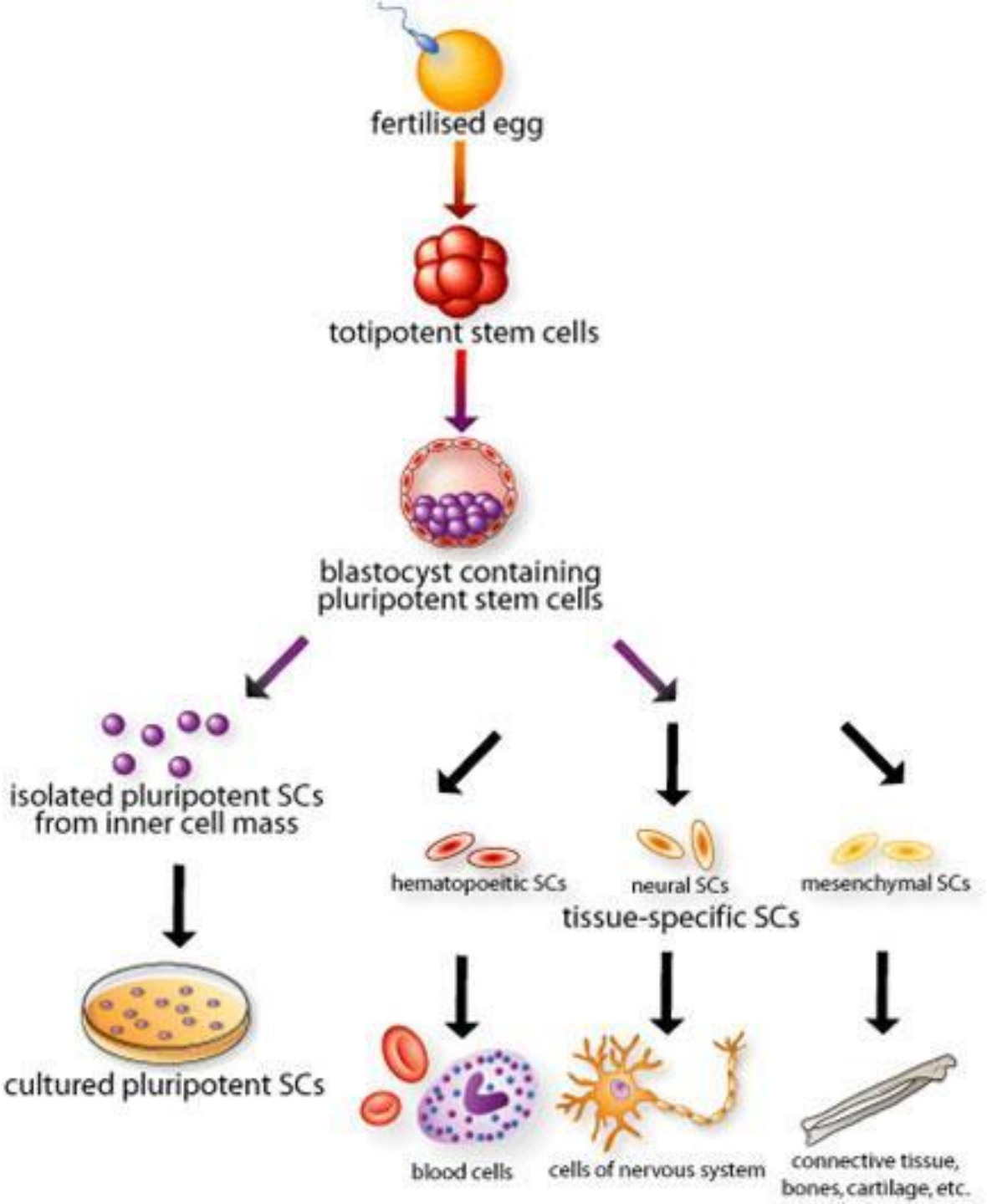
contact-inhibited
monolayer of
normal cells




multilayer of
uninhibited
cancer cells

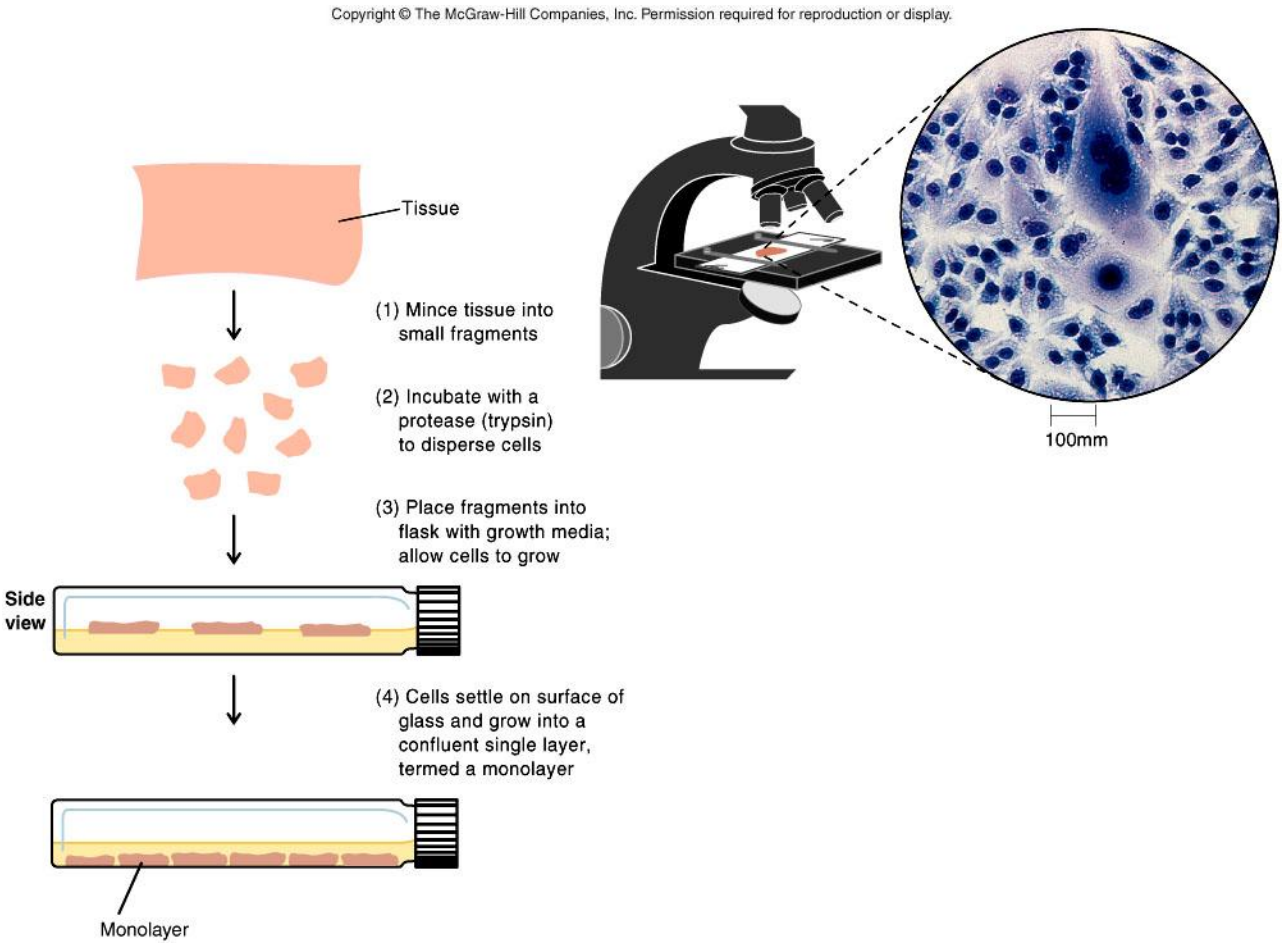
No contact inhibition & anchorage dependency–
Continuous cell line

| Finite cell line | Continuous cell line |
| --- | --- |
| Cell line that die after several sub cultures | Cell line that grow indefinitely |
| Cells grow through a limited number of generation and have a limited life | Obtained from transformed cells that divide rapidly and continuously |
| Cells grow slowly and form a monolayer | Cells form multiple layers |
| Doubling time ranges 24-96 hrs | Generation time 12-14 hrs |
| Exhibits anchorage dependent, Contact inhibition and density limitation | No contact inhibition, anchorage dependence and density limitation |


fertilised egg
totipotent stem cells
blastocyst containing
pluripotent stem cells
isolated pluripotent SCs
from inner cell mass
cultured pluripotent SCs
hematopoeitic SCs
neural SCs
mesenchymal SCs
tissue-specific SCs
blood cells
cells of nervous system
connective tissue,
bones, cartilage, etc.

# TYPES OF CULTURE

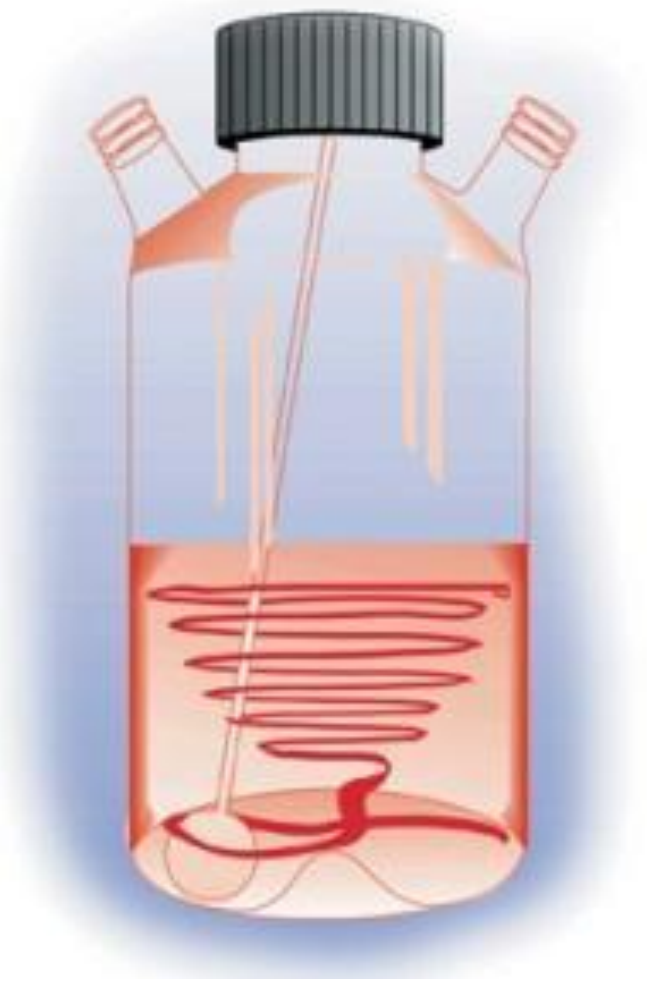
- MONOLAYER



- SUSPENSION CULTURE

1. Batch culture

2. Fed batch culture
3. Semi continuous
4. Perfusion
5. Continuous flow



# CULTURE MEDIA

- NATURAL

  - Biological fluids – Plasma clot, amniotic fluid, ascetic fluid, pleural fluid, serum, aqueous humour from eye, insect haemolymph

  - Tissue extract – Chick embryo, spleen, liver, bonemarroe extract etc

## ❖ ARTIFICIAL MEDIA

- With Serum – Eagles minimum essential medium with 5-20% serum

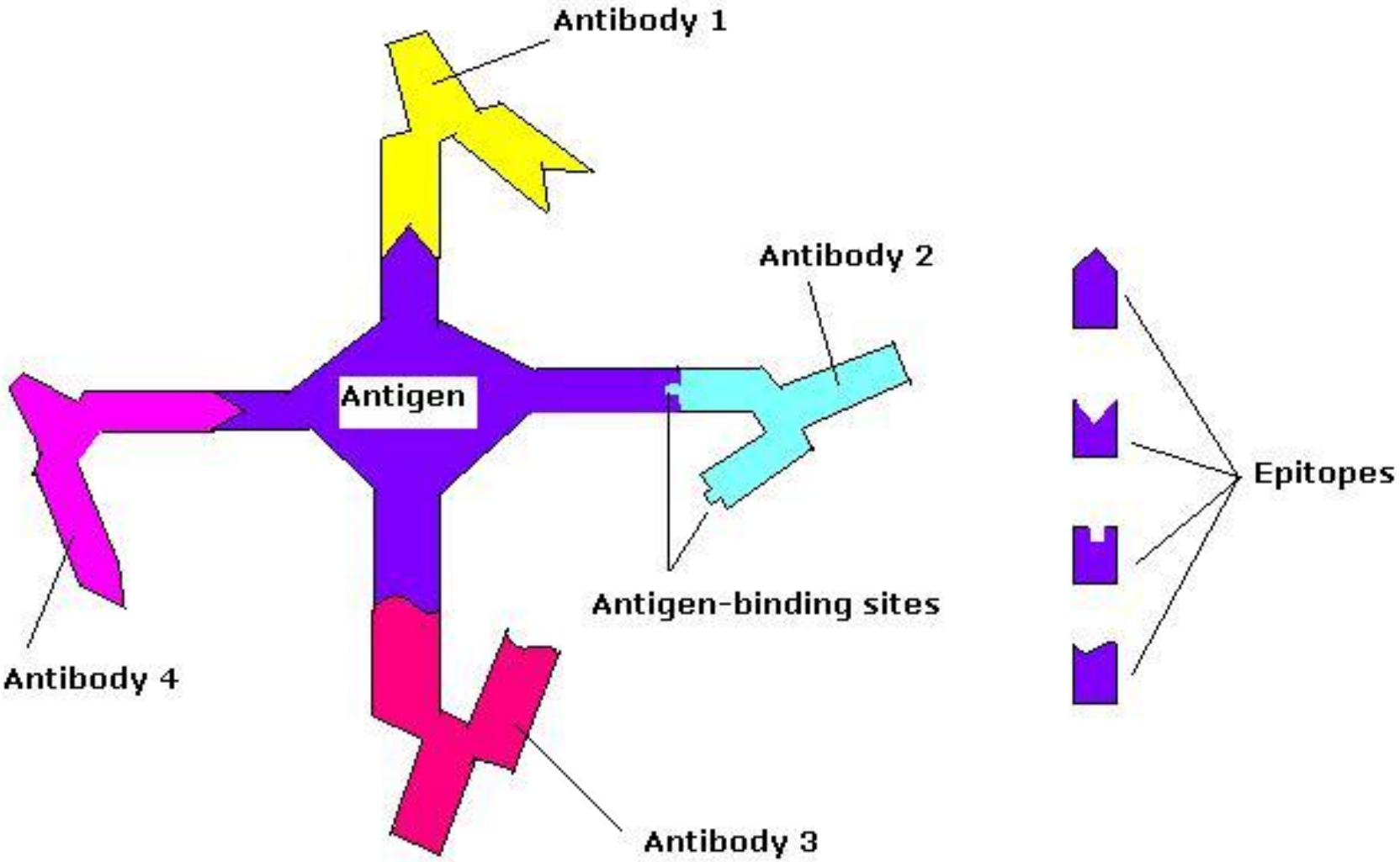
- Serum free medium – Dulbeccos enriched modification of Minimum Essential Medium (DME)

# Application

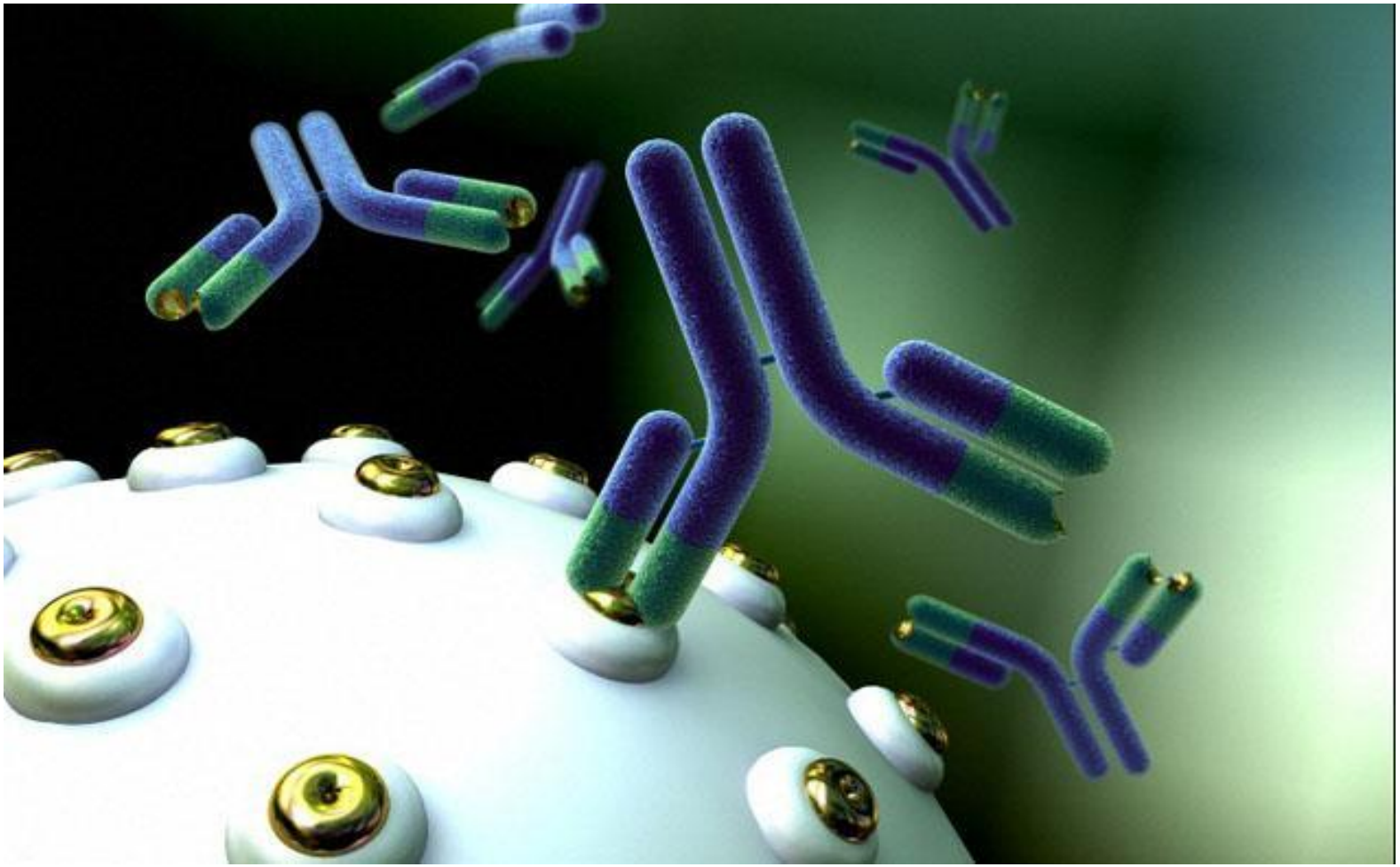
1. Production of Monoclonal Antibodies
2. Production of Interferons
3. Production of Vaccines
4. Large scale production of valuable products such as enzymes, hormones, interleukins etc.

# HYBRIDOMA TECHNOLOGY AND MONOCLONAL ANTIBODIES

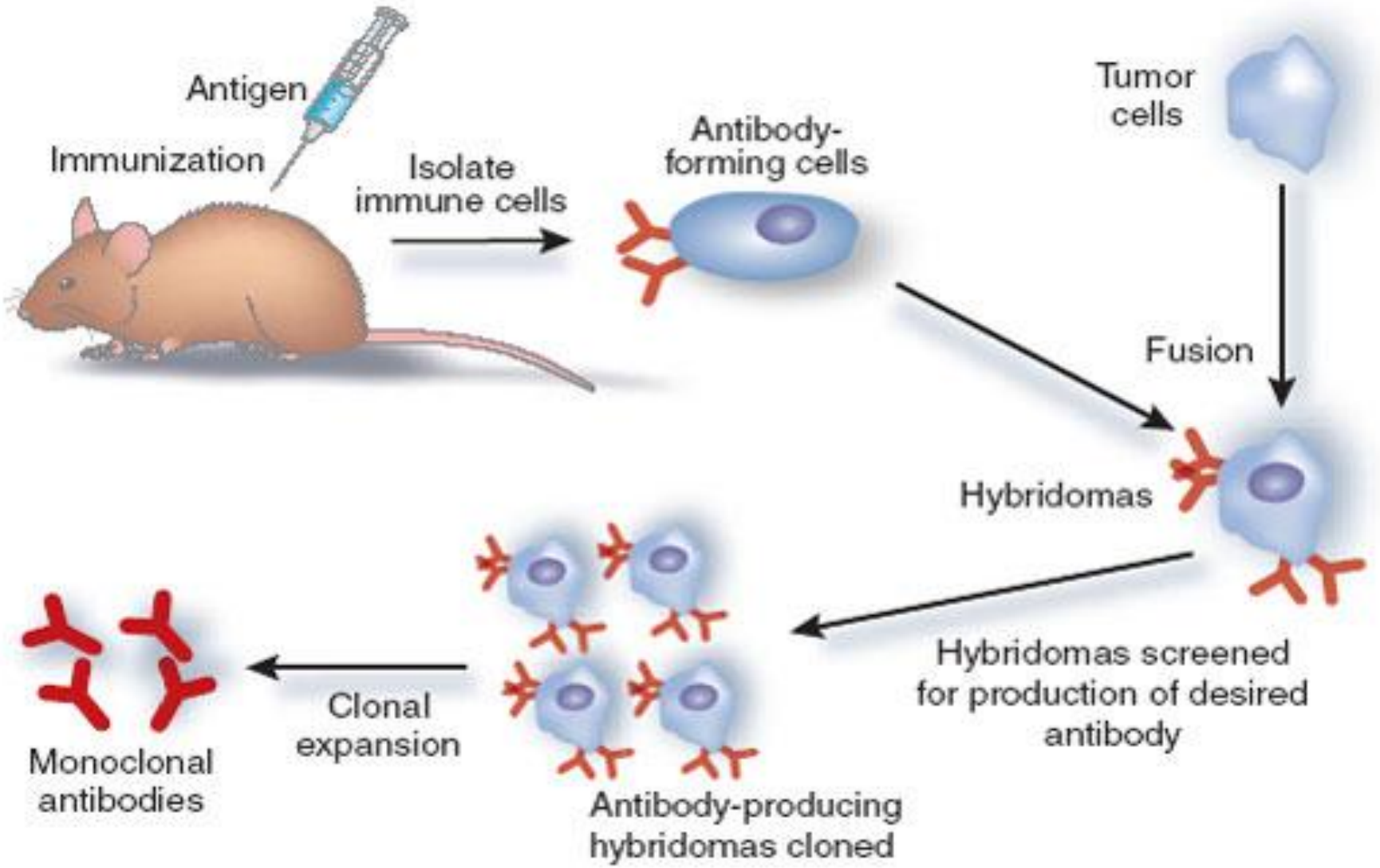
# POLYCLONAL ANTIBODIES

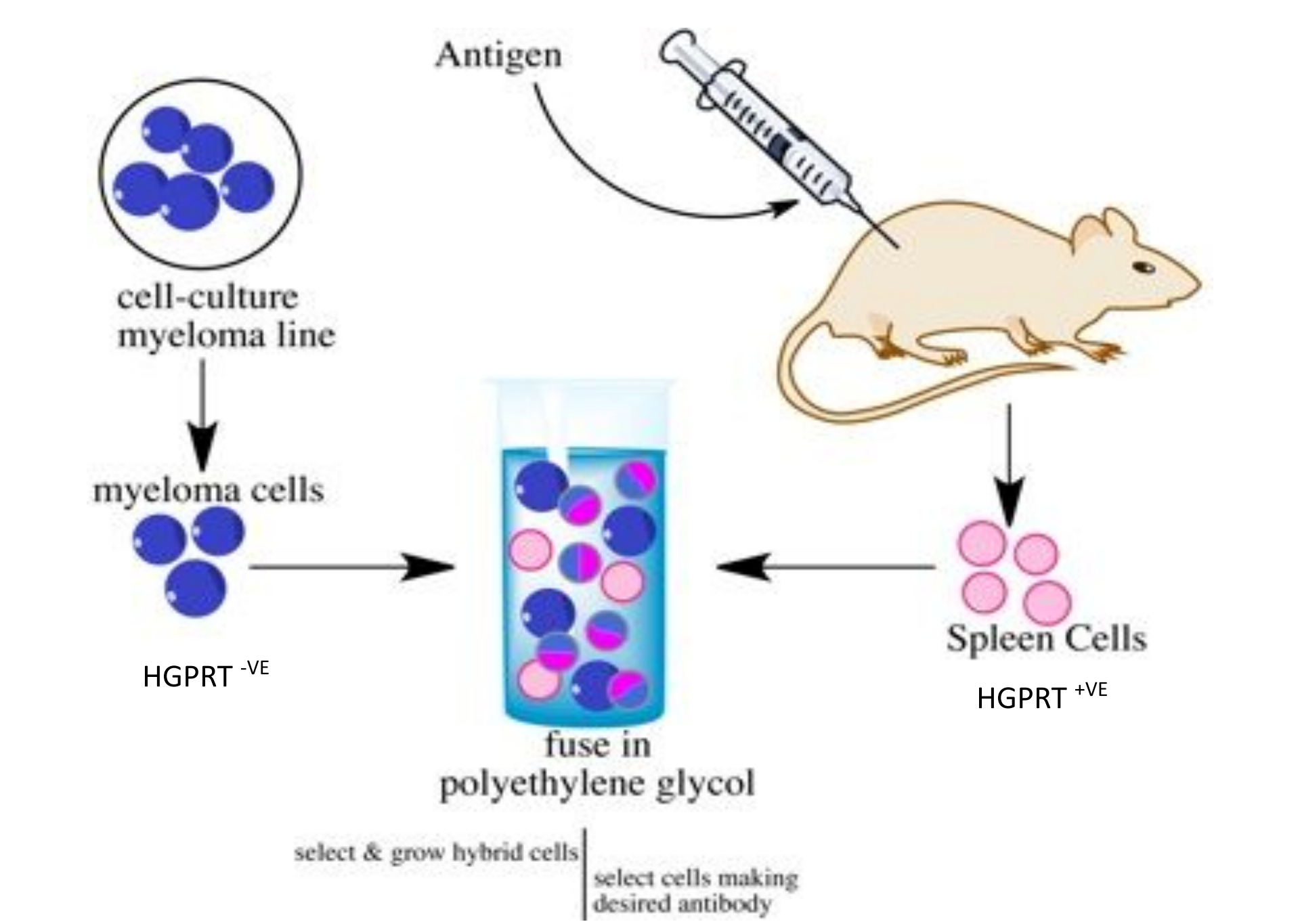


# MONOCLONAL ANTIBODIES



# Production of monoclonal antibodies - Hybridoma technology




Antigen
cell-culture
myeloma line
myeloma cells
HGPRT -VE
Spleen Cells
HGPRT +VE
fuse in
polyethylene glycol
select & grow hybrid cells
select cells making
desired antibody

# PATHWAYS INVOLVED IN NUCLEOTIDE SYNTHESIS

- DIHYDROFOLATE PATHWAY: Carried out when the cell is HGPRT$^{-ve}$ – Hypoxanthine Guanine Phospho Rybosyl Transferase

 Aminopteryne can block the pathway

- SALVAGE PATHWAY : Carried out when the cell is HGPRT$^{+ve}$

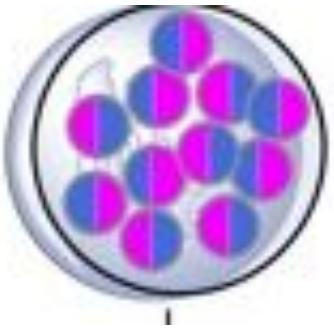
 Need Hypoxanthine and Thymine as source of purine and pyramidine

**Myeloma cells are HGPRT- and cannot create nucleotides in the salvage pathway.**

**Plasma cells are HGPRT+ and can utilize hypoxanthine in the salvage pathway.**

# Hybridoma selection (HAT media)

Myeloma cells



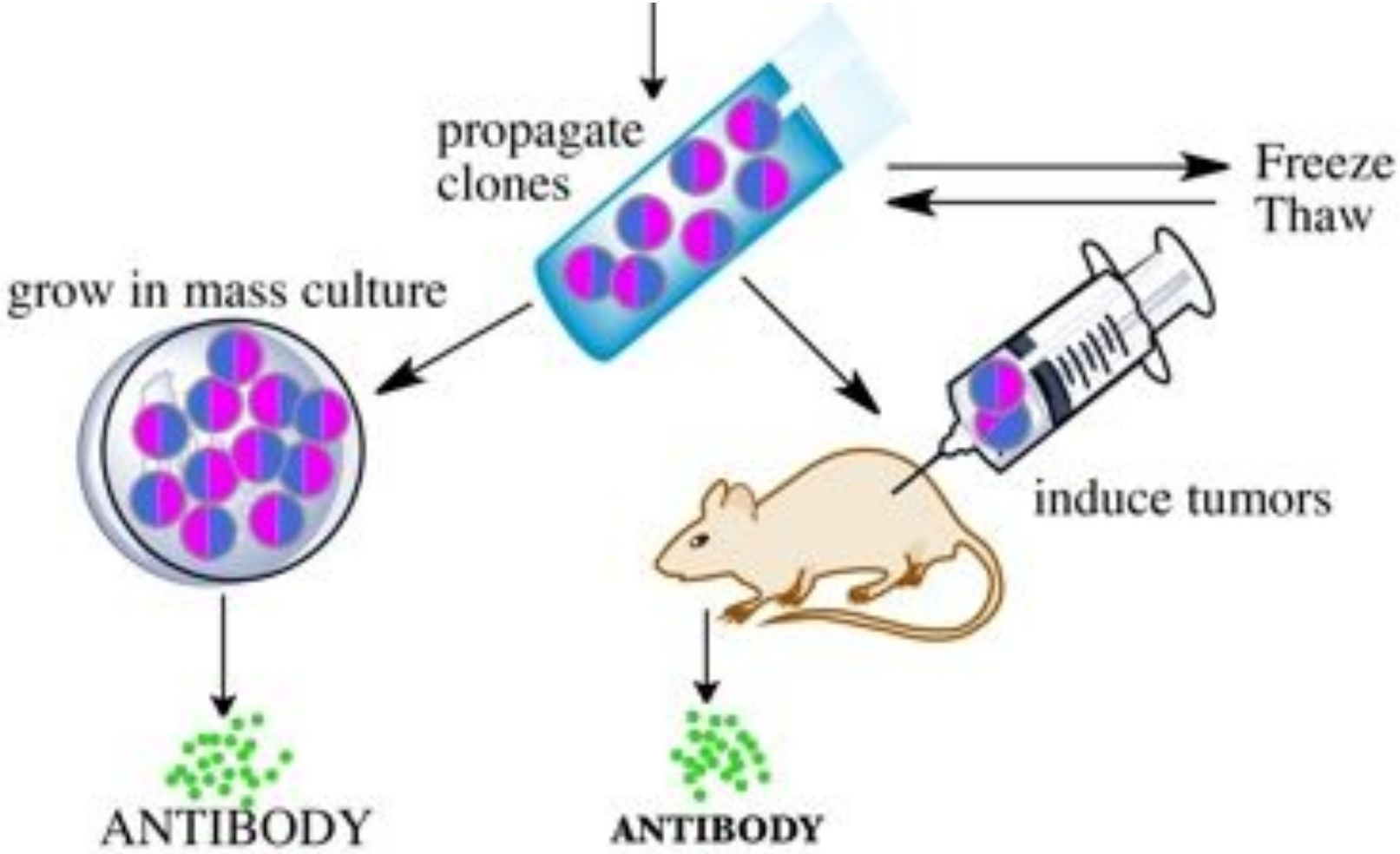
Hybrid cells


| | | | |
|---|---|---|---|
| **HAT fate:** | **DIES** | **SURVIVES** | **DIES** |
| **Explanation:** | **Unable to synthesize DNA:** | **Immortal and restored DNA synthesis:** | **Mortal:** |

**Myeloma cells : HGPRT $^{-ve}$ mutation cause loss of function in the 'Salvage Pathway'**
**Aminopteryne blocks the usual dihydrofolate pathway**

**Hybrid cells : HGPRT $^{+ve}$ and carries out the 'Salvage Pathway' due to spleen cells**
**Immortal due to fusion with myolma cells**

**Spleen cells : HGPRT $^{+ve}$ but eventually dies because of limited number of replication cycles**


propagate
clones
Freeze
Thaw
grow in mass culture
induce tumors
ANTIBODY
ANTIBODY

# Applications

1. Used to detect presence and quantity of antibody specific substances
2. Helps to detect antigen in immuno histochemistry
3. Helps to purify compounds in immunoprecipitation and affinity chromatography
4. Used to prevent tissue rejection in serological identification tests
5. Used to make immunotoxins for treatment of cancer

   [Monoclonal Ab + Toxin. Ab localise the target whereas toxin kills the Ag]

6. For accurate detection of cancer cells
7. Used in the classification of ABO blood groups and RH etc.
8. Diagnosing various diseases by ELISA, Agglutination, Precipitation etc.