B Pharmacy 6th sem.
Subject: Pharmaceutical Biotechnology
Subject Code: BP605T

INTRODUCTION TO BIOTECHNOLOGY

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Objective of Course

- Understanding the principle of biotechnology and importance of immobilized enzymes in pharamaceutical industries.
- Genetic engineering application in relation to production of pharmaceuticals

Learning Outcomes

- Students will learn about biotechnology with references to pharmaceutical sciences.
- They will learn about biosensor and various methods used for immobilization of enzymes.
- They will be able to implement the theoretical concept of genetic engineering and protein engineering.

Module-1

- Brief introduction to Biotechnology with reference to Pharmaceutical Sciences.
- Enzyme Biotechnology- Methods of enzyme immobilization and applications.
- Biosensors- Working and applications of biosensors in Pharmaceutical Industries.
- Brief introduction to Protein Engineering.
- Basic principles of genetic engineering.

What is biotechnology?

- Biotechnology = bios (life) + logos (study of or essence)
  - Literally ‘the study of tools from living things’

- CLASSIC: The word "biotechnology" was first used in 1917 to describe processes using living organisms to make a product or run a process, such as industrial fermentations. (Robert Bud, The Uses of Life: A History of Biotechnology)

- LAYMAN: Biotechnology began when humans began to plant their own crops, domesticate animals, ferment juice into wine, make cheese, and leaven bread (AccesExcellence)

What are the stages of biotechnology?

- Ancient Biotechnology
  - early history as related to food and shelter, including domestication
- Classical Biotechnology
  - built on ancient biotechnology
  - fermentation promoted food production
  - medicine
- Modern Biotechnology
  - manipulates genetic information in organism
  - genetic engineering

Modern biotechnology

- Cell biology
  - Structure, organization and reproduction
- Biochemistry
  - Synthesis of organic compounds
  - Cell extracts for fermentation (enzymes versus whole cells)
- Genetics
  - Resurrection of Gregor Mendel's findings 1866 1900s
    - Theory of Inheritance (ratios dependent on traits of parents)
    - Theory of Transmission factors
  - W.H. Sutton – 1902
    - Chromosomes = inheritance factors
  - T.H. Morgan – *Drosophila melanogaster*

Modern biotechnology

Molecular Biology

- Beadle and Tatum (Neurospora crassa)
  - One gene, one enzyme hypothesis
  - Charles Yanofsky colinearity between mutations in genes and amino acid sequence (*E. coli*)
  - Genes determine structure of proteins

- Hershey and Chase – 1952
- T2 bacteriophage – ^{32}P DNA, not ^{35}S protein is the material that encodes genetic information

Modern biotechnology

- Watson, Crick, Franklin and Wilkins (1953)
  - X-ray crystallography
  - 1962 – Nobel Prize awarded to three men
  - Chargaff – DNA base ratios
  - Structural model of DNA developed
- DNA Revolution – Promise and Controversy!!!
- Scientific foundation of modern biotechnology
  - based on knowledge of DNA, its replication, repair and use of enzymes to carry out in vitro splicing DNA fragments

What are the areas of biotechnology?

- Organismic biotechnology
  - uses intact organisms and does not alter genetic material
- Molecular Biotechnology
  - alters genetic makeup to achieve specific goals

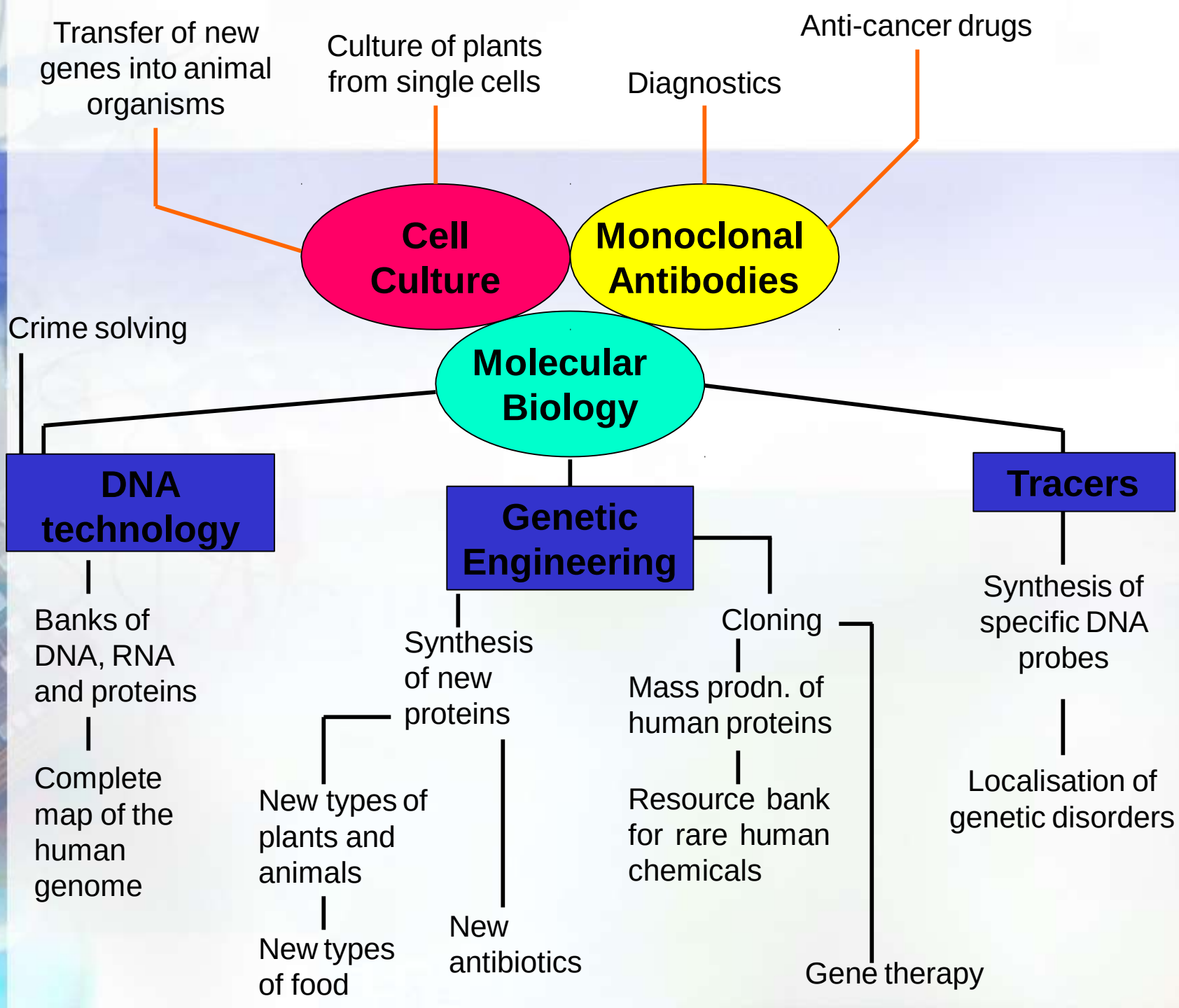
Transgenic organism: an organism with artificially altered genetic material

What are the benefits of biotechnology?

- Medicine
  - human
  - veterinary
  - biopharming
- Environment
- Agriculture
- Food products
- Industry and manufacturing

What are the applications of biotechnology?

- Production of new and improved crops/foods, industrial chemicals, pharmaceuticals and livestock
- Diagnostics for detecting genetic diseases
- Gene therapy (e.g. ADA, CF)
- Vaccine development (recombinant vaccines)
- Environmental restoration
- Protection of endangered species
- Conservation biology
- Bioremediation
- Forensic applications
- Food processing (cheese, beer)


Transfer of new genes into animal organisms
Culture of plants from single cells
Diagnostics
Anti-cancer drugs
Cell Culture
Monoclonal Antibodies
Crime solving
Molecular Biology
DNA technology
Genetic Engineering
Tracers
Banks of DNA, RNA and proteins
Complete map of the human genome
Synthesis of new proteins
New types of plants and animals
New types of food
New antibiotics
Cloning
Mass prodn. of human proteins
Resource bank for rare human chemicals
Gene therapy
Synthesis of specific DNA probes
Localisation of genetic disorders

ENZYME IMMOBILIZATION

What Is Enzyme Immobilization ?

Enzyme immobilization may be defined as a process of confining the enzyme molecules to a solid support over which a substrate is passed and converted to products.

What Is An Immobilized Enzyme?

An immobilized enzyme is one whose movement in space has been restricted either completely or to a small limited region.

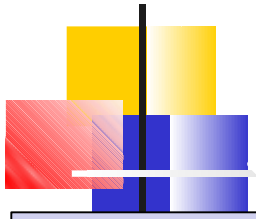
Why Immobilize Enzymes?

- Protection from degradation and deactivation.
- Re-use of enzymes for many reaction cycles, lowering the total production cost of enzyme mediated reactions.
- Ability to stop the reaction rapidly by removing the enzyme from the reaction solution.
- Enhanced stability.
- Easy separation of the enzyme from the product.
- Product is not contaminated with the enzyme.

An Ideal Carrier Matrices For Enzyme Immobilization

- Inert.
- Physically strong and stable.
- Cost effective.
- Regenerable.
- Reduction in product inhibition.

CLASSIFICATION OF CARRIERS



Inorganic Carriers

•High pressure stability.
•May undergo abrasion

Examples:

1.Commercialy SiO2 available materials-
○Porous glass.
○Silica.
2.Mineral materials - (clays)
Celite ,Centonite

Organic Natural Carriers

•Favourable compatibility with proteins.

Examples:

1.cellulose derivatives-
○DEAE-cellulose
○CM-cellulose.
2.Dextran.
3.Polysacharides
Agarose, Starch
Pectine ,Chitosan

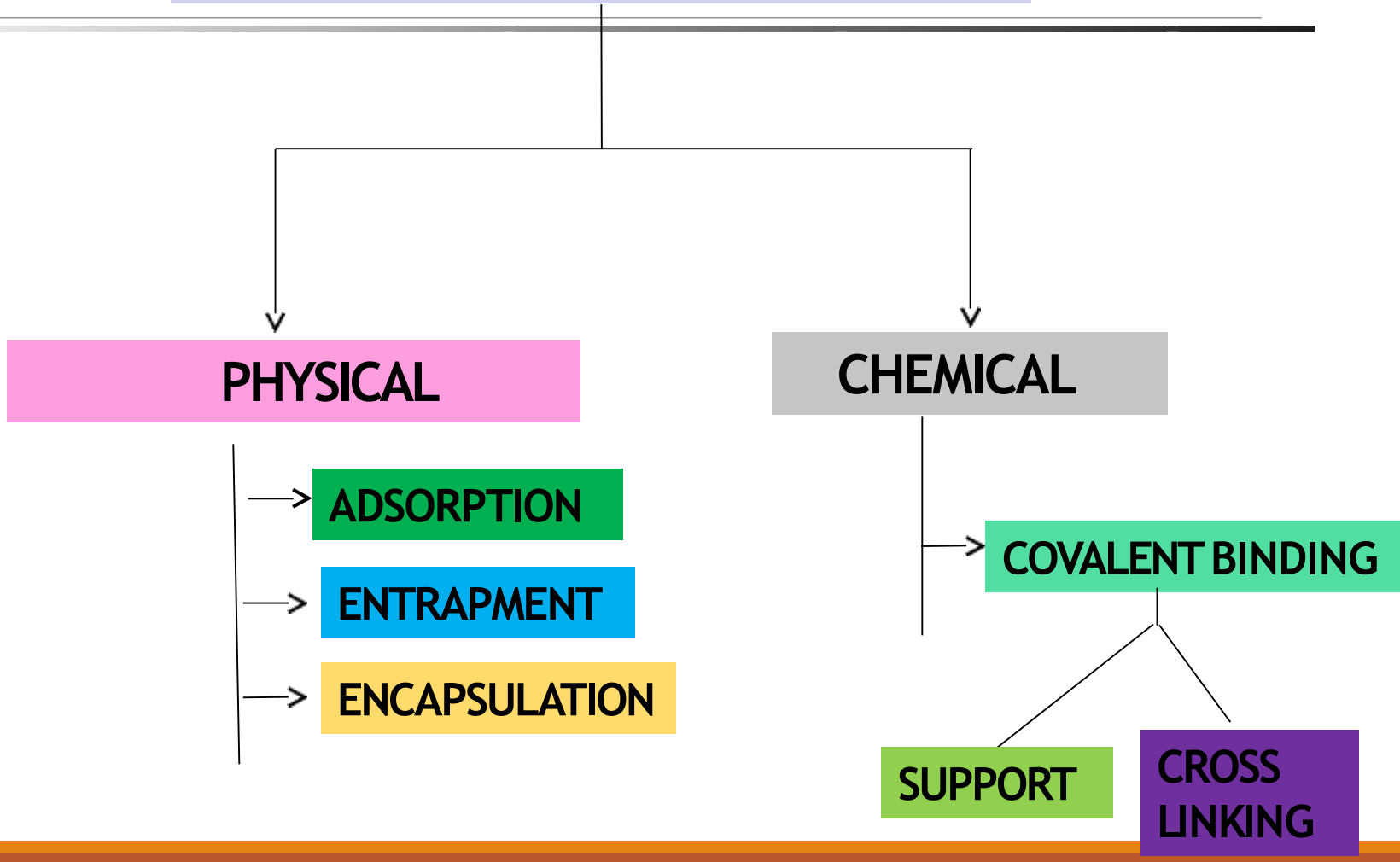
Organic Synthetic Carriers

•High chemical and mechanical stability.

Examples:

1.Polystyrene
2.Polyvinylacetate
3. Acrylic polymers

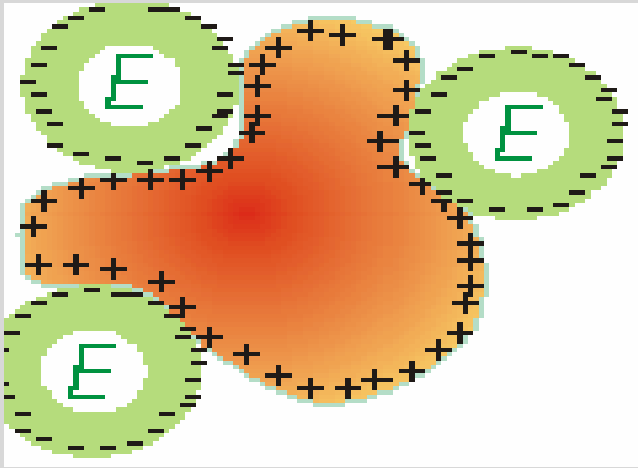
METHODS FOR ENZYME IMMOBILIZATION



Physical Methods For Immobilization

ADSORPTION

- Involves the physical binding of the enzyme on the surface of carrier matrix.
- Carrier may be organic or inorganic.
- The process of adsorption involves the weak interactions like Vander Waal or hydrogen bonds.



- Carriers: - silica, bentonite, cellulose, etc.
- e.g. catalase & invertase

ADVANTAGE

- 1. Simple and economical
- 2. Limited loss of activity
- 3.Can be Recycled, Regenerated & Reused.

DISADVANTAGES

- 1.Relatively low surface area for binding.
- 2.Exposure of enzyme to microbial attack.
- 3.Yield are often low due to inactivation and desorption.

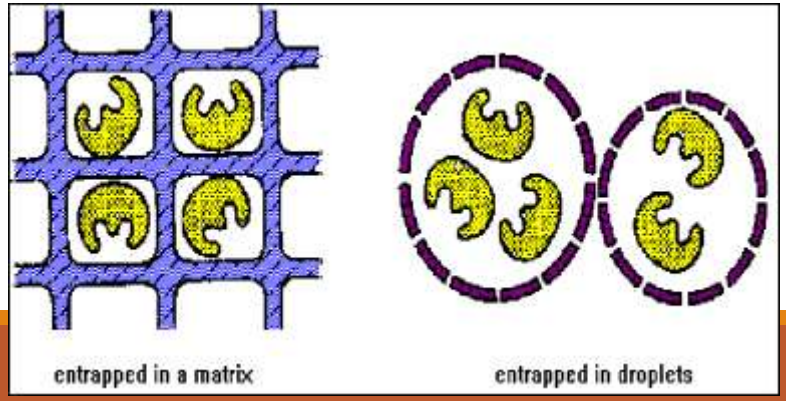
Entrapment

- In entrapment, the enzymes or cells are not directly attached to the support surface, but simply trapped inside the polymer matrix.
- Enzymes are held or entrapped within the suitable gels or fibres.
- It is done in such a way as to retain protein while allowing penetration of substrate. It can be classified into lattice and micro capsule types.

Inclusion in gels: Poly acrylamide gel, Poly vinyl alcohol gels.

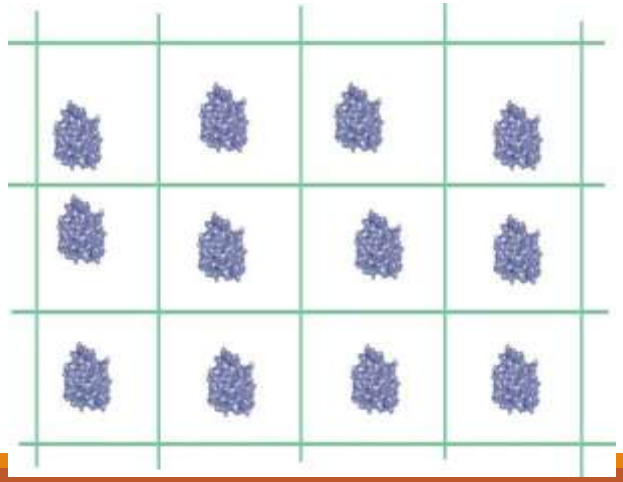
Inclusion in fibers: Cellulose and Poly -acryl amide gels.

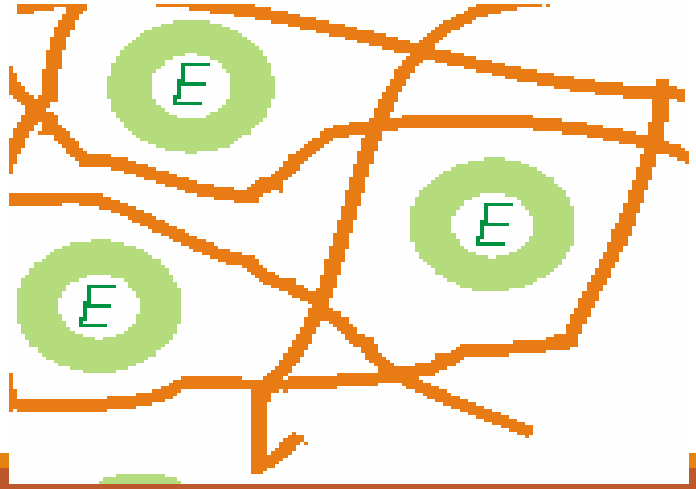
Inclusion in micro capsules: Polyamine, Polybasic - acid chloride monomers.



Lattice-Type Entrapment

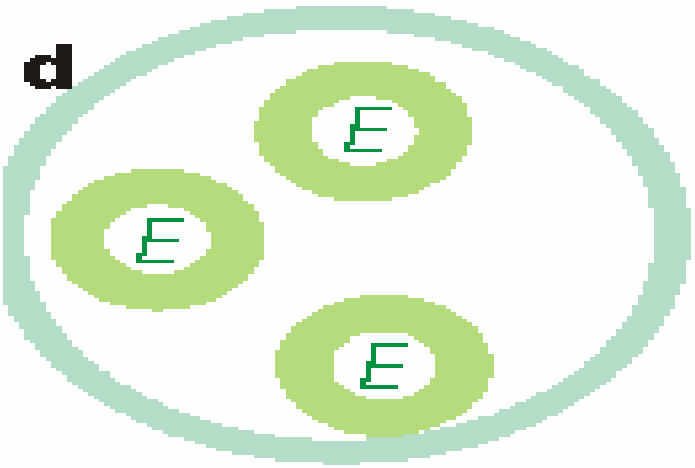
- Entrapment involves entrapping enzymes within the interstitial spaces of a cross-linked water-insoluble polymer. Some synthetic polymers such as *polyarylamide*, *polyvinylalcohol*, *etc*... and natural polymer (starch) have been used to immobilize enzymes using this technique.





MicrocapsuleType Entrapmet/ Encapsulation/Membrane confinement

- It involves enclosing the enzymes within semi -permeable polymer membranes e.g. semi permeable collodion or nylon membranes in the shape of spheres.



ADVANTAGES DISADVANTAGS

- 1.No chemical modification.
- 2.Relatively stable forms.
- 3.Easy handling and re-usage.

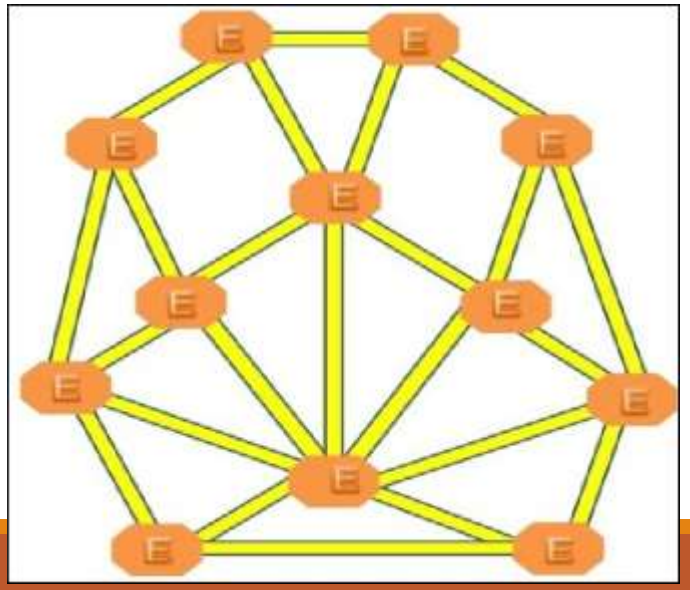
- 1. The enzyme may leak from the pores.

Covalent Binding

- Based on the binding of enzymes and water-insoluble carriers by covalent bonds
- The functional groups that may take part in this binding are Amino group, Carboxyl group, Sulfhydryl group, Hydroxyl group, Imidazole group, Phenolic group, Thiol group, etc
- Disadvantages : covalent binding may alter the conformational structure and active center of the enzyme, resulting in major loss of activity and/or changes of the substrate
- Advantages : the binding force between enzyme and carrier is so strong that no leakage of the enzymes occurs, even in the presence of substrate or solution of high ionic strength.

Cross Linking

- Cross linking involves intermolecular cross linking of enzyme molecules in the presence/absence of solid support.
- The method produces a 3 dimensional cross linked enzyme aggregate (insoluble in water) by means of a multifunctional reagent that links covalently to the enzyme molecules.



Advantages of cross linking:-

1. Very little desorption(enzyme strongly bound)
2. Higher stability (i.e. ph, ionic & substrate concentration)

Disadvantages of cross linking:-

1. Cross linking may cause significant changes in the active site.
2. Not cost effective.

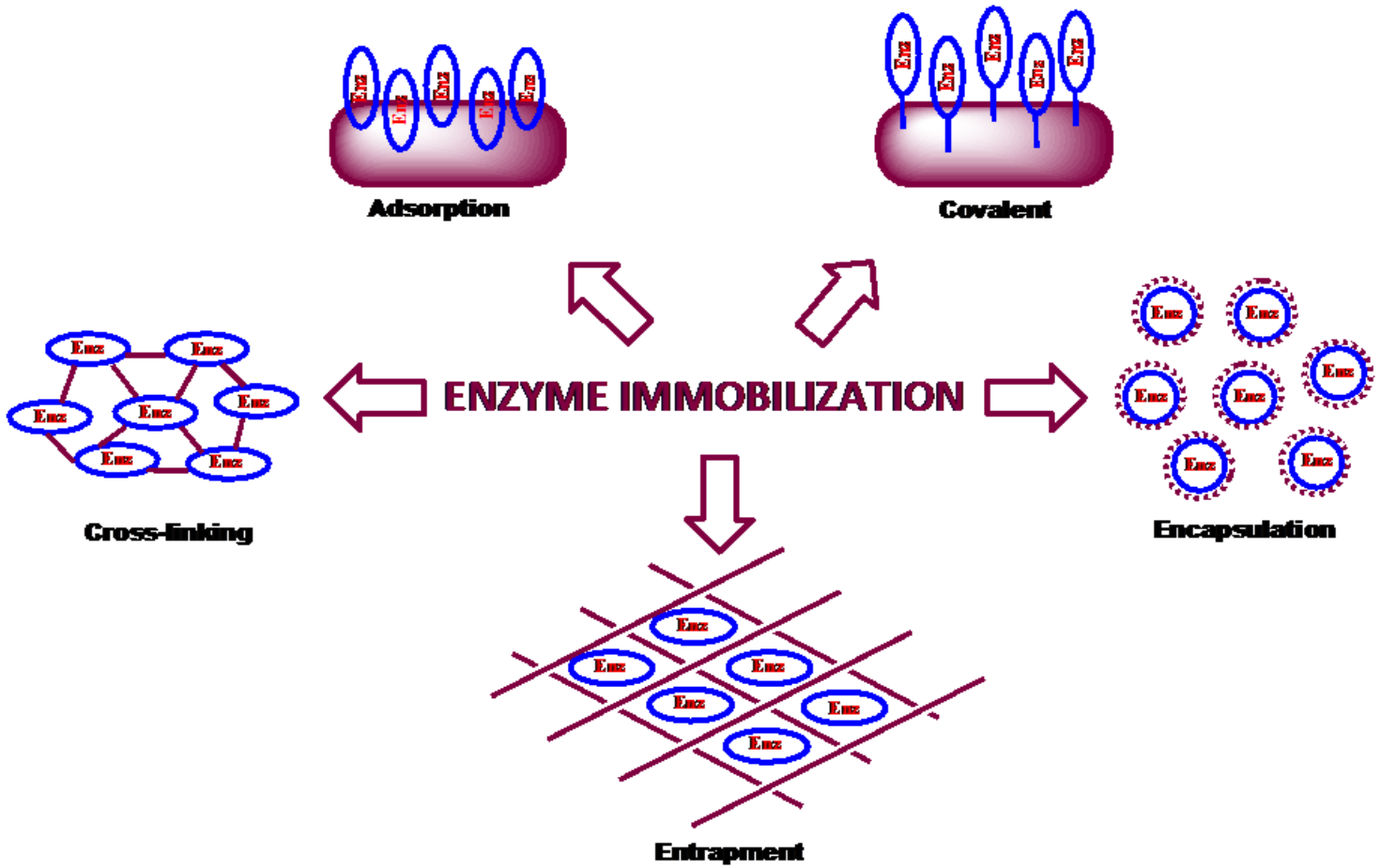


Fig. Pictorial representation of different immobilization methods.

Comparison Between The Methods

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

Limitations Of Enzyme Immobilization

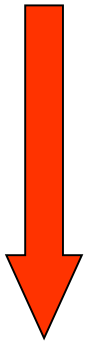
- Cost of carriers and immobilization.
- Changes in properties (selectivity).
- Mass transfer limitations.
- Problems with cofactor and regeneration.
- Problems with multienzymes systems.
- Activity loss during immobilization.

BIOSENSOR

What is a Biosensor?

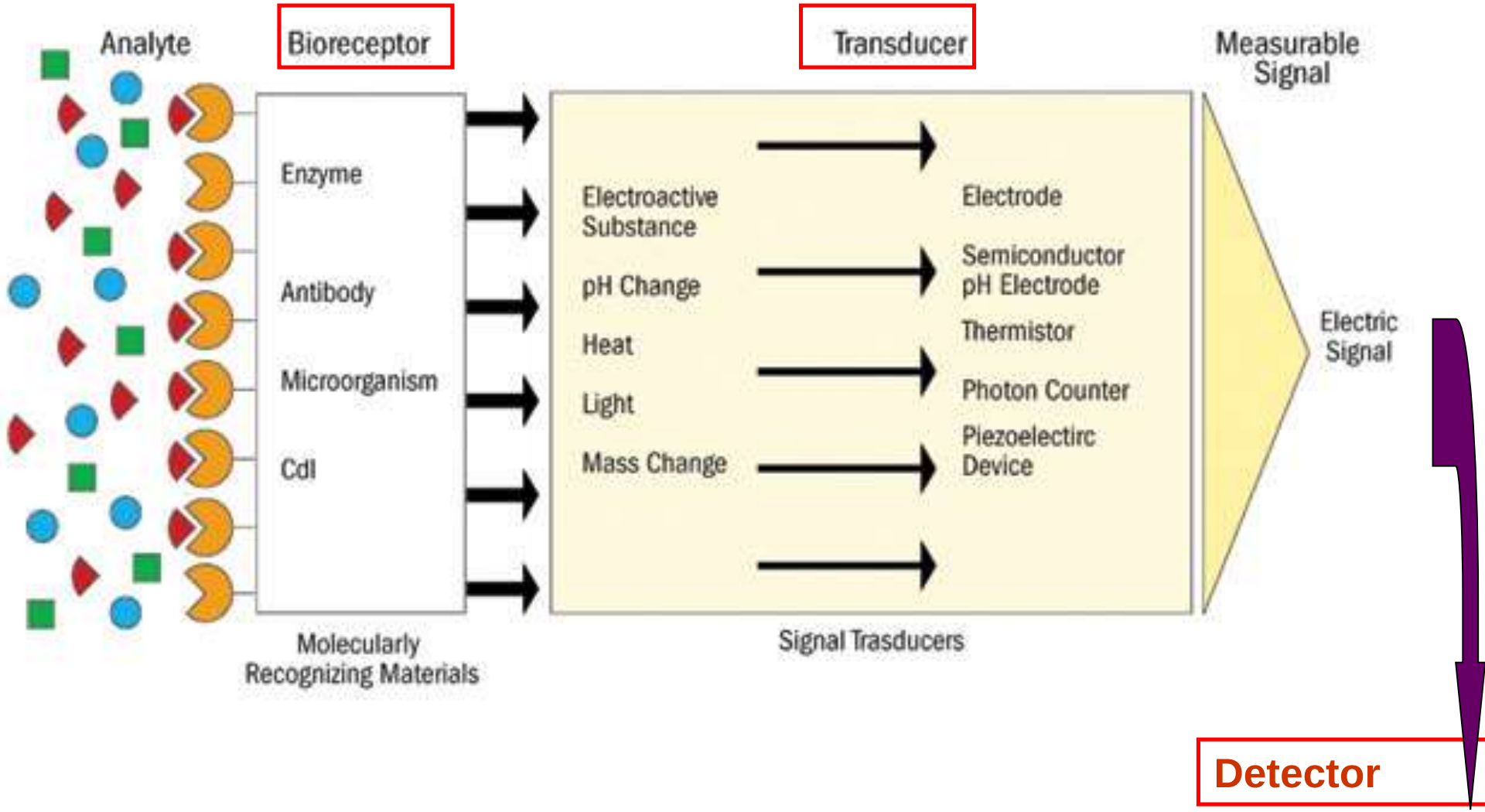
A biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element (IUPAC, 1996)

1) Biosensor ≠ Bioanalytical system



2) An enzyme electrode is a biosensor

Components of a Biosensor



Basic Characteristics of a Biosensor

1. LINEARITY	Linearity of the sensor should be high forthe detection of high substrate concentration.
2. SENSITIVITY	Value of the electrode response per substrate concentration.
3. SELECTIVITY	Chemicals Interference must be minimised for obtaining the correct result.
4.RESPONSE TIME	Time necessary for having 95% of the response.

Biosensor

1. The Analyte *(*What do you want to detect)

Molecule - Protein, toxin, peptide, vitamin, sugar, metal ion

2. Sample handling (How to deliver the analyte to the sensitive region?)

(Micro) fluidics - Concentration increase/decrease), Filtration/selection

Biosensor

❖3. Detection/Recognition

(How do you specifically recognize the analyte?)

4. Signal

(How do you know there was a detection)

Typical Sensing Techniques for Biosensors

- ✓Fluorescence
- ✓DNA Microarray
- ✓SPR Surface plasmon resonance
- ✓Impedance spectroscopy
- ✓SPM (Scanning probe microscopy, AFM, STM)
- ✓QCM (Quartz crystal microbalance)
- ✓SERS (Surface Enhanced Raman Spectroscopy)
- ✓Electrochemical

Types of Biosensors

1. **Calorimetric Biosensor**
2. **Potentiometric Biosensor**
3. **Amperometric Biosensor**
4. **Optical Biosensor**
5. **Piezo-electric Biosensor**

PIEZO-ELECTRIC BIOSENSORS

Piezo-electric devices use gold to detect the specific angle at which electron waves are emitted when the substance is exposed to laser light or crystals, such as quartz, which vibrate under the influence of an electric field.

ELECTROCHEMICAL BIOSENSORS

- **For applied current: Movement of e- in redox reactions detected when a potential is applied between two electrodes.**

Potentiometric Biosensor

- **For voltage: Change in distribution of charge is detected using ion-selective electrodes, such as pH-meters.**

OPTICAL BIOSENSORS

- **Colorimetric for color**

Measure change in light adsorption

- **Photometric for light intensity**

Photon output for a luminescent or fluorescent process can be detected with photomultiplier tubes or photodiode systems.

CALORIMETRIC BIOSENSORS

If the enzyme catalyzed reaction is exothermic, two thermistors may be used to measure the difference in resistance between reactant and product and, hence, the analyte concentration.

Electrochemical DNA Biosensor

- **Steps involved in electrochemical DNA hybridization biosensors:**
  - **Formation of the DNA recognition layer**
  - **Actual hybridization event**
  - **Transformation of the hybridization event into an electrical signal**

DNA biosensor

Motivated by the application to clinical diagnosis and genome mutation detection

Types DNA Biosensors

Electrodes

Chips

Crystals

Application of Biosensor

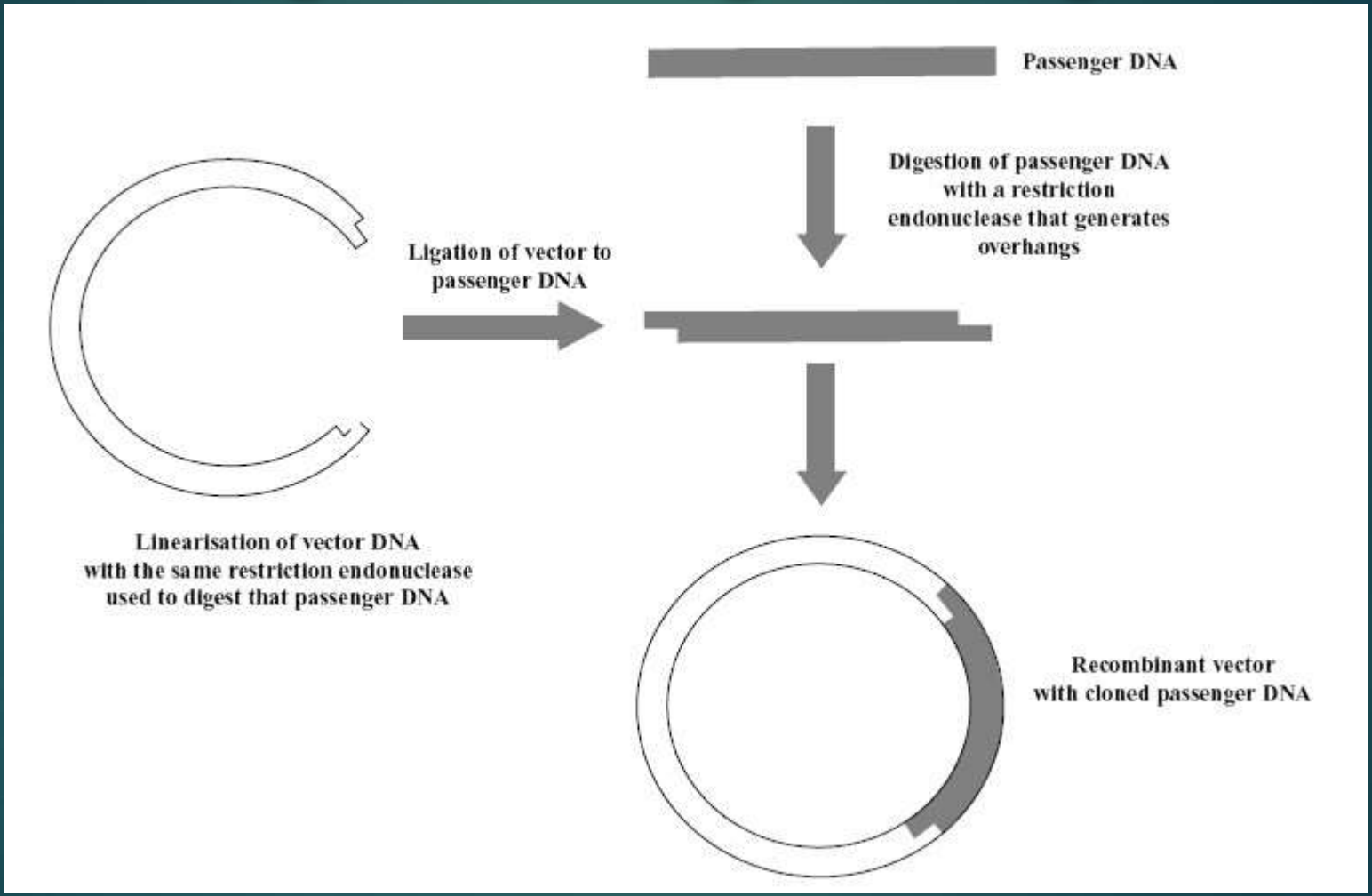
- Food Analysis
- Study of biomolecules and their interaction
- Drug Development
- Crime detection
- Medical diagnosis (both clinical and laboratory use)
- Environmental field monitoring
- Quality control
- Industrial Process Control
- Detection systems for biological warfare agents
- Manufacturing of pharmaceuticals and replacement organs

GENETIC ENGINEERING

- Genes are the fundamental basis of all life, determine the properties of all living forms of life, and are defined segments of DNA.
- Because DNA structure and composition in all living forms is essentially the same, any technology that can isolate, change or reproduce a gene is likely to have an impact on almost every aspect of society.

- Genetic engineering has been defined as the formation of new combinations of heritable material by the insertion of nucleic acid molecules, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur, but in which they are capable of continued propagation.

basic concept of genetic engineering



- gene technology is the modification of the genetic properties of an organism by the use of recombinant DNA technology.
- Genes may be viewed as the biological software and are the programs that drive the growth, development and functioning of an organism.
- By changing the software in a precise and controlled manner, it becomes possible to produce desired changes in the characteristics of the organism.

- These techniques allow the splicing of DNA molecules of quite diverse origin, and, when combined with techniques of genetic transformation etc., facilitate the introduction of foreign DNA into other organisms.
- The foreign DNA or gene construct is introduced into the genome of the recipient organism host in such a way that the total genome of the host is unchanged except for the manipulated gene(s).

- While traditional plant and animal genetic breeding techniques also change the genetic code it is achieved in a less direct and controlled manner.
- Genetic engineering will now enable the breeder to select the particular gene required for a desired characteristic and modify only that gene.
- Although much work to date has involved bacteria, the techniques are evolving at an astonishing rate and ways have been developed for introducing DNA into other organisms such as yeasts and plant and animal cell cultures.

- Life forms containing ‘foreign’ DNA are termed ***transgenic***
- These methods potentially allow totally new functions to be added to the capabilities of organisms, and open up vistas for the genetic engineering of industrial microorganisms and agricultural plants and animals that are quite breathtaking in their scope.
- This is undoubtedly the most significant new technology in modern bioscience and biotechnology.

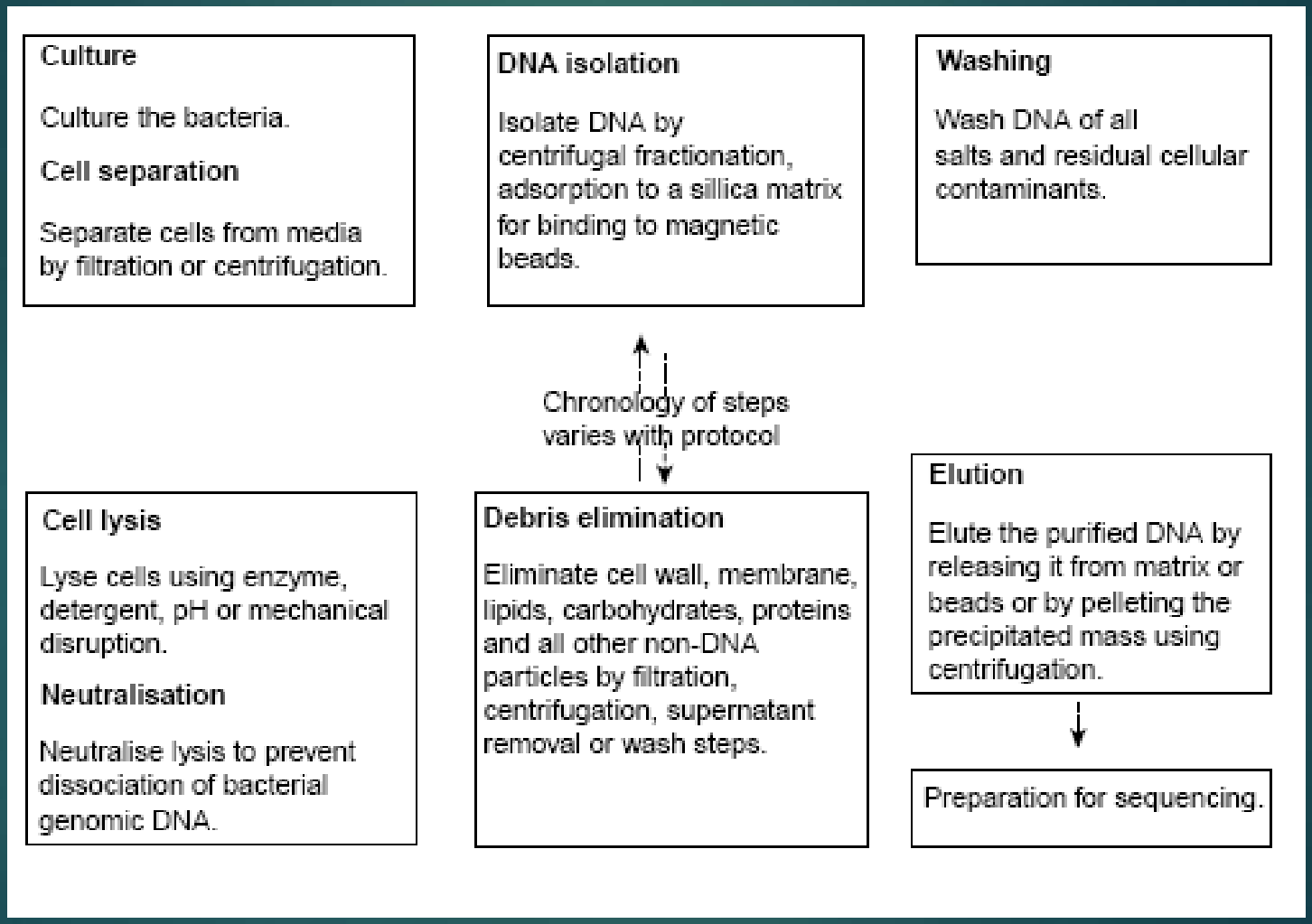
Applications

- In industrial microbiology it will permit the production in microorganisms of a wide range of hitherto unachievable products such as human and animal proteins and enzymes such as insulin and chymosin (rennet)
- in medicine, better vaccines, hormones and improved therapy of diseases;
- in agriculture, improved plants and animals for productivity, quality of products, disease resistance, etc;

- in food production, improved quality, flavour, taste and safety;
- in environmental aspects, a wide range of benefits such as pollution control can be expected.
- In microbial technology these techniques will be widely used to improve existing microbial processes by improving stability of existing cultures and eliminating unwanted side products.
- However, there are many who view genetic engineering as a transgression of normal life processes that goes well beyond normal evolution.

- Genetic engineering holds the potential to extend the range and power of almost every aspect of biotechnology.
- It is confidently anticipated that within this decade recombinant DNA techniques will form the basis of new strains of microorganisms with new and unusual metabolic properties.
- In this way fermentations based on these technical advances could become competitive with petrochemicals for producing a whole range of chemical compounds, for example ethylene glycol (used in the plastics industry) as well as improved biofuel production.
- In the food industry, improved strains of bacteria and fungi are now influencing such traditional processes as baking and cheese-making and bringing greater control and reproducibility of flavour and texture.

- A full understanding of the working concepts of recombinant DNA technology requires a good knowledge of molecular biology.
- The basic molecular techniques for the in vitro transfer and expression of foreign DNA in a host cell (*gene transfer technology*), including isolating, cutting and joining molecules of DNA, and inserting into a vector (carrying) molecule that can be stably retained in the host cell, were first developed in the early 1970s.


Culture
Culture the bacteria.
Cell separation
Separate cells from media by filtration or centrifugation.
DNA isolation
Isolate DNA by centrifugal fractionation, adsorption to a silica matrix for binding to magnetic beads.
Washing
Wash DNA of all salts and residual cellular contaminants.
Chronology of steps varies with protocol
Cell lysis
Lyse cells using enzyme, detergent, pH or mechanical disruption.
Neutralisation
Neutralise lysis to prevent dissociation of bacterial genomic DNA.
Debris elimination
Eliminate cell wall, membrane, lipids, carbohydrates, proteins and all other non-DNA particles by filtration, centrifugation, supernatant removal or wash steps.
Elution
Elute the purified DNA by releasing it from matrix or beads or by pelleting the precipitated mass using centrifugation.
Preparation for sequencing.

Cutting DNA molecules:

- DNA can be cut using mechanical or enzymatic methods.
- The non-specific mechanical shearing will generate random DNA fragments
- In contrast, when specific restriction endonuclease enzymes are used it is possible to recognise and cleave specific target base sequences in double-stranded (ds) DNA.

- Large numbers of different restriction endonucleases have been extracted and classified from a wide variety of microbial species.
- Restriction endonucleases are named according to the species from which they were first isolated, e.g. enzymes isolated from *Haemophilus influenzae* strain Rd are designated *Hind* and when several different restriction enzymes areisolated from the same organism they are designated *HindI*, *HindII* etc.

Splicing DNA:

- DNA fragments can be joined together in vitro by the action of specific DNA ligases.
- The DNA ligase that is widely used was encoded by phage T4.
- The composite molecules in which DNA has been inserted have also been termed ‘DNA chimaeras’

The vector or carrier system:

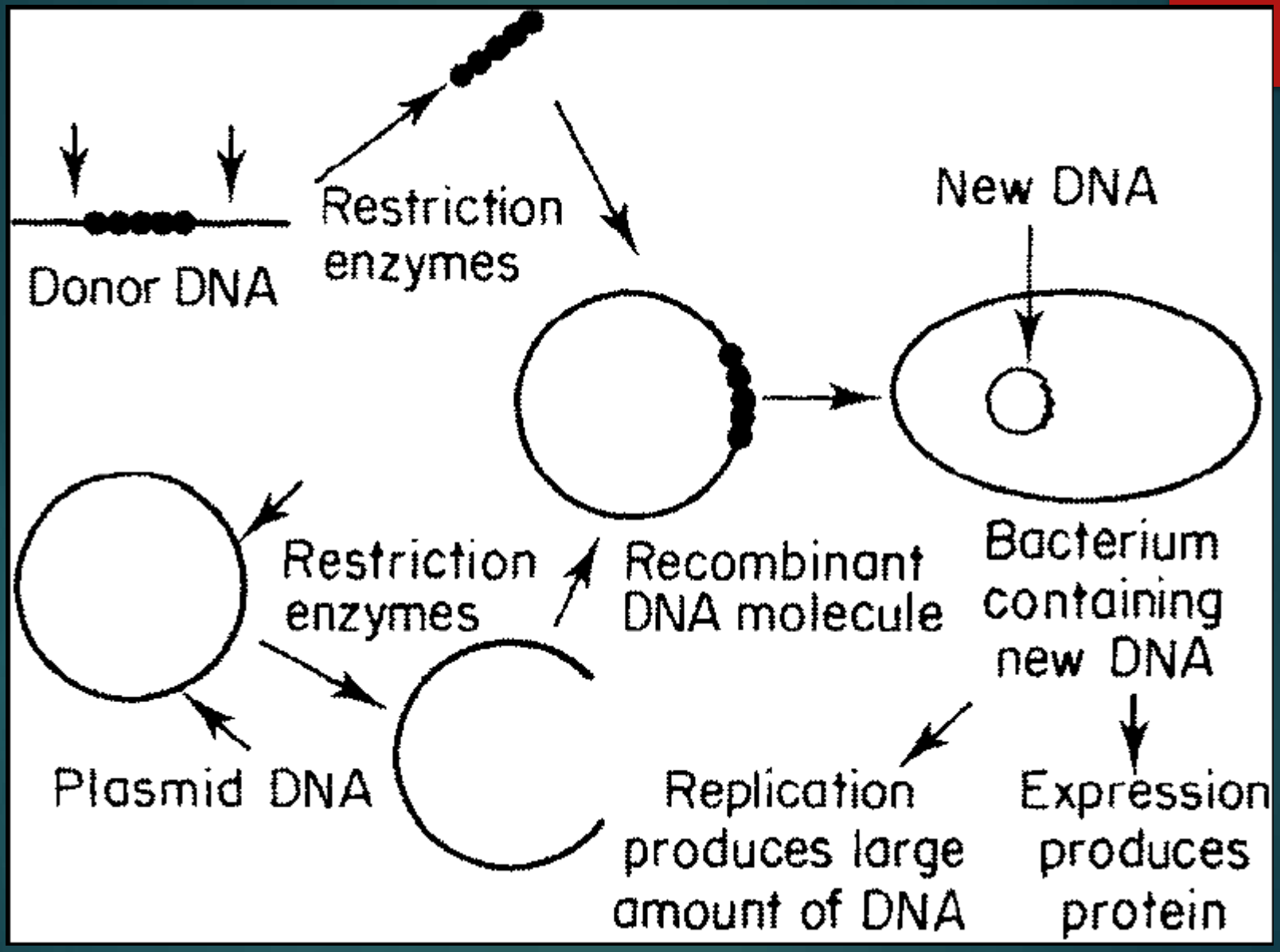
- Two broad categories of expression vector molecules have been developed as vehicles for gene transfer, *plasmids* (small units of DNA distinct from chromosomes) and b*acteriophages* (or bacterial viruses).
- Vector molecules should be capable of entering the host cell and replicating within it.
- Ideally, the vector should be small, easily prepared and must contain at least one site where integration of foreign DNA will not destroy an essential function.

Introduction of vector DNA recombinants:

- The new recombinant DNA can now be introduced into the host cell and if acceptable the new DNA will be cloned with the propagation of the host cell.
- Novel methods of ensuring DNA uptake into cells include *electroporation* and *mechanical particle delivery* or *biolistics*.

- Electroporation is a process of creating transient pores in the cell membrane by application of a pulsed electric field.
- Creation of such pores in a membrane allows introduction of foreign molecules, such as DNA, RNA, antibodies, drugs, etc., into the cell cytoplasm.
- Development of this technology has arisen from synergy of biophysics, bioengineering and cell and molecular biology.
- While the technique is now widely used to create transgenic microorganisms, plants and animals, it is also being increasingly used for application of therapeutics and gene therapy.

- The mechanical particle delivery or ‘gene gun’ methods deliver DNA on microscopic particles into target tissue or cells. This process is increasingly used to introduce new genes into a range of bacterial, fungal, plant and mammalian species and has become a main method of choice for genetic engineering of many plant species including rice, corn, wheat, cotton and soybean.


Donor DNA
Restriction enzymes
New DNA
Bacterium containing new DNA
Recombinant DNA molecule
Restriction enzymes
Plasmid DNA
Replication produces large amount of DNA
Expression produces protein

Strategies involved in genetic engineering

Formation of DNA fragments	Extracted DNA can be cut into small sequences by specific enzymes, restriction endonucleases, found in many species of bacteria.
Splicing of DNA into vectors	The small sequences of DNA can be joined or spliced into the vector DNA molecules by an enzyme DNA ligase creating an artificial DNA molecule.
Introduction of vectors into host cells	The vectors are either viruses or plasmids, and are replicons and can exist in an extrachromosomal state; transfer normally by transduction or transformation.
Selection of newly acquired DNA	Selection and ultimate characterisation of the recombinant clone.

Thanks