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B. Pharm
Semester-V

Subject Name: Pharmaceutical Biotechnology
Subject Code: BP505TT

Chapter-1
BIOTECHNOLOGY & PROTEIN ENGINEERING

Introduction: "Biotechnology" by using scientific methods with organisms to produce new products or new forms of organisms.

- any technique that uses living oraganisms or substance from those organisms to make or modify a product, to improve plants or animals, or to devlop micro-oraganisms for specific uses.
- Manipulation of gene is called genetic enginerrig or recombinant DNA technology.
- Genetic engineering involves taking one or more genes from a location in one organism and either.
- Transferring them to another oraganism.
- Putting them back into another oraganism.
- Putting them back into the original organism in different combinations.

Applications of Biotechnology:

- cell and molecular biology.
- Microbiology.
- Genetics.
- Anatomy and physiology.
- Biochemistry.
- Engineering.
- Computer science.
- Virues-resistance crap plants and livestock.
- Therapies that used gene to cure diseases.
- Recombinant vaccines to prevent disease.

Computer in biotechnology:

- Computer modeling may be done before it is tested with animals.

Classical Biotechnology Advances:

- Today many thing s are produced.
- 1) pharmaceutical compounds such as antibiotics.
- 2) many chemicals, hormones, and pigments.
- 3) enzyme with large varity of uses.
- 4) biomass for commercial and animal consumption.

Application:

- 1) Agriculture: plant bredding to improve resistance to pests, diseases drought and salt conditions.
- 2) Chemical Industry: production of bulk, chemicals and solvents such as ethanol, citric acid, acetone and butanol.
- 3) Medicine: development of novel therapeutica molecules for medical treatments
- Diagnostics, drug delivery systems, tissue engineering replicating organs, gene therapy.

- 4) Food industry: production of bakers' yeast, cheese, yougurt and fermented foods such as vinegers and soy sauce.
- Wine making process
- Production of coloring and flavors agents.

FUTURE OF MEDICINE:

- Smart drugs for cancer and autoimmune diseases.
- Gene-based diagnostics and therapies.
- Stem cells and regenerative medicine.
- Health and longevity.

WHAT IS ENZYME IMMOBILIZATION?

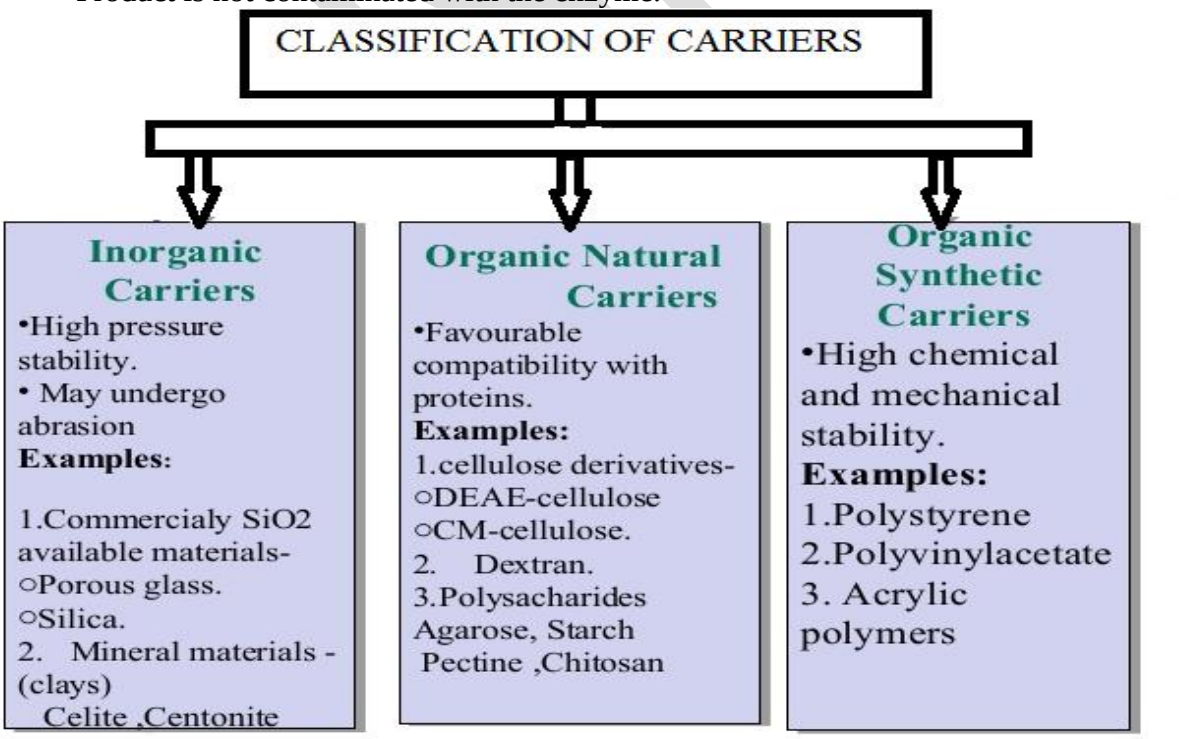
- It is defined as a process of confining the enzyme molecules to a solid support over which a substrate is passed and converted to products.

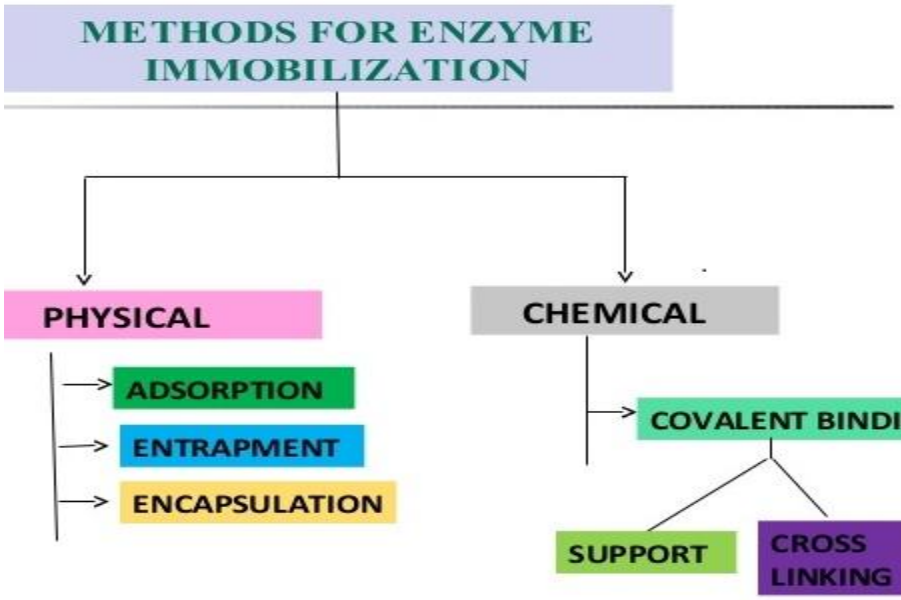
WHAT IS AN IMMOBILIZATION ENZYME?

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

WHY IMMOBILIZATION 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.

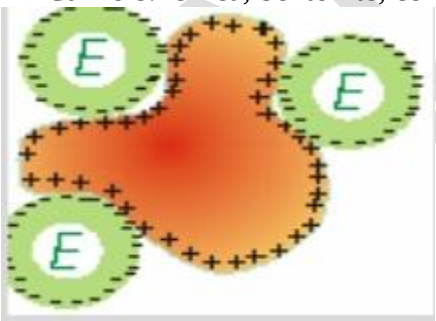




Physical method for Immobilization:

1) ADSORPTION:

- Involves the physical binding of the enzyme on the surface if carrier matrix.
- Carrier may be organic or inorganic.
- The process of adsorption involves the weak interaction like vander waal or hydrogen bonds.
- Carriers:- silica, bentonite, cellulose.



Advantages:

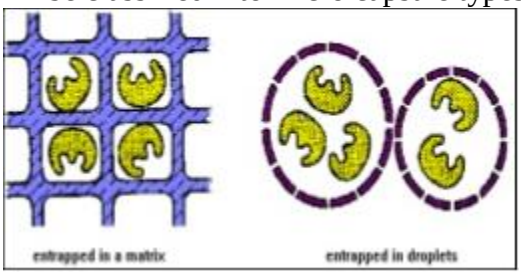
1) Simple and economical
2) Limited loss of activity
3) Can be recycled, reused.

Disadvantage:

1) Relatively low surface area for binding.
2) Exposure of enzyme to microbial attack.

3) 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 fibers.
- It is done in such a way as to retain protein while allowing penetration of substrate, it can be classified into micro capsule types.



Advantage:

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

Disadvantage:

- The enzyme may leak from the process.

4) MICROCAPSUL ENTRAPMENT:

- It involves enclosing the enzymes within semi-permeable polymer membranes.

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

5) COVALENT BONDING:

- 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.

- Disadvantages: covalent bonding may alter the conformational structure and active enter of the enzyme, resulting in major loss of activity and changes of the substrate.
- Advantages: the binding between and carrier is so strong that no leakage of the enzymes occurs, even in the presence of substrate or solution of high ionic strength.

- **LIMITATIONS OF ENZYME IMMOBILIZATION:**

- Cost of carriers and immobilization.

- Changes in properties.

- Activity loss during immobilization

- Mass transfer limitation.

- **APPLICATION OF ENZYME IMMOBILIZATION IN PHARMACY:**

- Production of antibiotics.

- Production of steroids.

- Production of amino acids

- Production of acids

- Production of organic compounds.

- Analytical application.

PRODUCTION OF ENZYME

- Introduction:
- Enzyme are macromolecules biological catalysts .
- Enzyme accelerate or catalyze chemical reactions.
- The molecules at the beginning of the process are called substrates and the enzyme converts these into different molecules, called product.
- **Type Of Enzymes:**
- 1) ADAPTIVE:-Produced only when the need arises. E.g when a cell is deficient of particular nutrients.
- 2) CONSTITUTIVE:- produced always irrespective the amount of substrate.

HISTORY:

- The first enzyme produced industrially was the "**fugal amalyse takadiastase**" which was employed as a pharmaceutical agent for digestive disorders.
- By 1969, 80% of all laundry detergents contained enzymes, chiefly proteases.
- Due to occurrence of allergies among the production workers & consumers, the sale of such enzyme utilizing detergents decrease.
- **Location of enzyme**:-
- Enzyme which are produced within the cell or cytoplasmic membrane are called as endocellular enzyme.

APPLICATION:

- Optimization of fermentation condition.
- New cell breaking methods like homogenizer, sonication etc.
- Modern purification processes like counter current distribution, ion- exchange chromatography.
- Immobilization of enzymes.
- Continuous enzyme production.

METHOD OF ENZYME PRODUCTION:

- **1) SEMISOLID CULTURE**
- **2) SUBMERGED CULTURE**
- 1) Semisolid culture:-
- The enzyme producing culture is grown on the surface of a suitable semi-solid substrate.

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- Preparation of production medium – bran is mixed with solution containing nutrient salts.

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- PH is maintained at a neutral level. Medium is steam sterilized in an autoclave while stirring

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- The sterilized medium is spread on metal trays up to a depth of 1-10 cm.

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- Culture is inoculated either in the autoclave after cooling or in trays.
- High enzyme concentration in a crude fermented material.

Enzymes produced by semi-solid culture :-

enzyme	**micro – organisms**
ά- amylase	aspergillus oryzae
Lactose	A. orycae
protease	A. niger

- **Advantage Of Semi-solid Culture** :-
- It involves comparatively low investment.
- Allows the mould to grow into their natural state.
- **Disadvantage Of Semi-solid Culture** :-
- Requires more space & more labor.
- Involves greater risk of infection.
- Difficult to introduce automation in such systems.

Submerged Culture:-

- Fermentation equipment used is the same as in the manufacture of antibiotics.
- It's cylindrical tank of stainless steel
- It's equipped with an agitator.
- A cooling system & various ancillary equipment
- Good growth is not enough to obtain a higher enzyme yield.
- Presence of inhibitors or inducers should also be checked in the medium.
- **E.g.** presence of lactose induce the production of β- galactosidase.
- As inducer are expensive, constitutive mutant are used which do not require an inducer.
- Certain surfactant in the production medium ↑es the yield of certain enzyme.

Advantage of submerged culture:

- Requires less labor & space

- Low risk of infection
- Automation is easier.

Disadvantage :

- Initial investment cost is very high.

AMYLASE:

- Amylase is an enzyme that catalyse the hydrolysis of starch in to sugar.
- Present in saliva of humans.
- Hydrolysis of starch with amylase will first result in the formation of a short polymer dextrin and than the disaccharide maltose and finally glucose.
- Glucose is not sweet as fructose thus the next step would be the conversion of glucose to fructose by the enzyme glucose isomers.
- Types Of Amylase :
- 1) ά - amylase
- 2) β – amylase
- 3) ϒ – amylase.

ά - AMYLASE :-

- Also called as glucan glucanohydrolase.
- Calcium metallo enzymes which can not function in absence of calcium ions.
- Break down long carbohydrate chains of amylose & amyloceptin.
- Amylase is broken down to maltose molecules.
- Found in saliva & pancreas.
- Found in plants, fungi & bacteria.
- Because it can act anywhere on the substrate, alpha - amylase tends to be faster acting than beta - amylase.

β – AMYLASE:

- Also called as 1,4,alpha,D – glucan maltohydrate synthesized by bacteria, fungi & plants.
- During the ripening of fruit , β- amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit.
- The optimum PH for β – amaylase is 4.0 -5.0.

ϒ – amylase:

- Also termed as glucan 1,4 alpha- glucosidase.
- Cleaves alpha(1-6) glucosidc linkages as well as the last alpha (1-4) glucosidc linkages at the non reducing end of amylase & amylopectin, yielding glucose.

- The ϒ – amylase has most acidic optimum PH of all amylase because it is most activated around PH 3.
- Effect of α- amylases :
- Starch: breakdown the starch polymer but liquefying does not give free sugar.
- Saccharogenic :- gives free sugar

APPLICATION:-

- production of sweeteners for the food industry.
- Removal of starch sizing from woven cloth.
- Liquefaction of starch pastes which are formed during the heating steps In the manufacture of corn & chocolate syrups.
- Production of bread & removal of food spots in the dry cleaning industry.

LIPASES:

- Lipases are also called as glycerol ester hydrolyses
- They are subclass of esterase's
- It splits fat into fatty acid.
- They are extracellular enzyme
- Bacteria producing lipase include species of pseudomonas.

APPLICATION:

- Primarily marketed for therapeutic purpose as digestive enzymes.
- Since free fatty acids affect the odor and taste of cheese and the cheese ripening process is affected by lipases.
- In the soap industry, lipase from Candida cylindraceae is used to hydrolyze oils.

PECTINASES:

- Pectinases is enzyme that berks down pectin into a polysaccharide found in plant cell walls.
- Pectic enzyme includes pectolyase, pectozenzyme & polygalacturonase.
- Pectin is jelly like structure which helps cement plant cells together & in which other cell wall components, such as cellulose fibrils are embedded.
- Basic structure of a pectin consists of α1,4 linked galactonronic acid with upto 95 % of its carboxyl groups esterified with methanol.
- Pectinase might typically be activated at 45 to 55 and well at a PH of 3.0 to 6.5
- Application:
- Pectinase enzymes are commonly used in process involving the degradation of plant material such as speeding up the extraction of fruit juice from fruit.
- It also used in wine production since the 1960.
- Helps to clarify fruit juices and maceration of vegetables & fruits.

PROTEASES:

- Protease is enzyme that performs the hydrolysis of peptide bonds.
- Peptides bonds links the amino acids to give the final structure of a protein.
- Proteinases are extracellular & peptidases are endocellular. This is the second most important enzyme produced on a large scale after amylase.

CLASSIFICATION BASED UPON THE RESIDUES IN THE CATALYTIC SITE.

- 1) Serine Protease
- 2) Threonine Protease
- 3) Aspartate Protease
- 4) Cysteine Protease
- 5) Glutamatic Acid Protease
- 6) Metalloprotease

CLASSIFICATION BASED UPON THE PH IN WHICH THE PROTEASES ARE ACTIVE

- 1) alkaline serine proteases
- 2) acid proteases
- 3) natural proteases
- Alkaline serine proteases:-
- PH of the production medium is kept at 7.0 for satisfactory results
- Optimum temp. maintained is 30 to 40℃
- Enzymes used in detergents are chiefly protease from bacillus strains. (bacillopeptidase)
- These enzyme are not inhibited by EDTA but are inhibited by DFP (di isopropyl fluorophosphate)
- PROTEASES FOR THE USE IN DETERGENT INDUSTRIES:
- Stability at high temperature
- Stability in alkaline rang (PH 9 to 11)
- Stability in association with chelating agents
- But self life is affected in presence of surface active agents.

SCREENING

- Because the enzyme should be stable in alkaline condition, screening for better producedure is done by using highly alkaline media.
- Genetic manipulation can also be carried out.
- Fermentation process:
- Cultures are stored in the lyophilized state or under liquid nitrogen.
- Initial culture are carried out in shaken flasks & small ferments at 30-37℃
- Highly oxygen partial pressure is generally necessary for optimal proteases titers.
- Time for fermentation is 48 to 72hr or depending upon the organism.

- Proteases must be converted in a particulate form before they are added to detergents.

NEUTRAL PROTEASES

- They are relatively unstable and calcium, sodium and chloride must be added for maximum stability.
- Not stable at higher temperature
- Producing organisms are B. sabtilis, B. megaterium.
- They are quickly inactivated by alkaline proteases.

ACID PROTEASES

- Similar to mammalin pepsin. (give rise to extraordinary quantities of immune reactive neurotensin (NT)
- It consists of rennin like proteases from fungi which are chiefly used in cheese production.
- They are used in medicine, in the digestion of soy protein for soya sauce production & to breakdown wheat gluten in the baking.

CATALASE

- The enzyme catalase is known to catalyse the breakdown of hydrogen peroxide into oxygen and water.
- Hydrogen peroxide metabolism is mainly regulated by this enzyme.
- Catalase is a common enzyme found in nearly all living organisms. It has been used as an important enzyme in much biotechnological area including bioremediation.

APPLICATION

- Used in food industry
- Used in detergent industry
- Used in milk industry

PENICILLINASE

- Gram +ve bacteria normally release β-lactamase to outside of the cell that will cleave penicillin before reaching the bacteria.
- Penicillin has to reach plasma membrane where the transpeptidase present to do its antibacterial action.

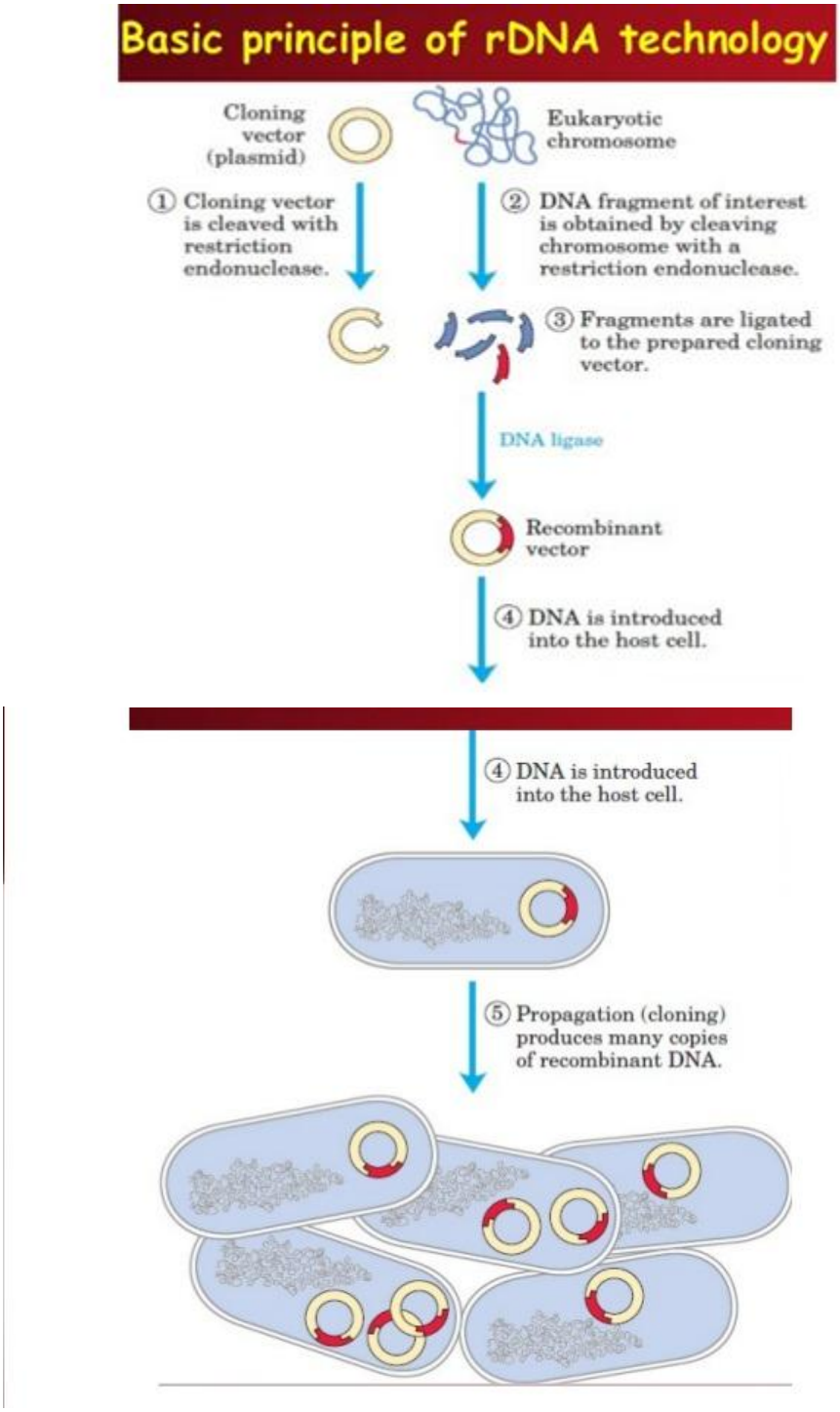
GENETIC ENGINEERING

INTRODUCTION

- Genetic recombination technology consists of the breakage and joining of DNA molecules.
- Genetically engineered DNA prepared by transplanting genes from one species into the cells of a host organism of a different species.
- Genetic engineering primarily involves the manipulation of genetic material to achieve the desire goal in pre determined way.

BASIC PRINCIPLE OF RECOMBINANT DNA TECHNOLOGY

- Manipulation and altering of genes
- Artificially copying a piece of DNA from one organism and joining this copy of DNA into the DNA of another organism.


Basic principle of rDNA technology
Cloning vector (plasmid)
Eukaryotic chromosome
① Cloning vector is cleaved with restriction endonuclease.
② DNA fragment of interest is obtained by cleaving chromosome with a restriction endonuclease.
③ Fragments are ligated to the prepared cloning vector.
DNA ligase
Recombinant vector
④ DNA is introduced into the host cell.
④ DNA is introduced into the host cell.
⑤ Propagation (cloning) produces many copies of recombinant DNA.

MOLECULAR TOOLS OF GENETIC ENGINEERING:

- The genetic engineer's tool kit or molecular tool namely the ENZYMES are most commonly used in recombinant DNA experiments.
- Restriction endonucleases – DNA cutting enzyme
- DNA ligase – DNA joining enzyme.
- Restriction enzymes act as molecular scissors and cut DNA at specific sites called restriction sites.

CLONING VECTORS:

- Vectors are the DNA molecule, which can carry a foreign DNA fragment to be cloned. They are self replicating in an appropriate host cell.
- The most important vectors are plasmids.
- An ideal characteristic of vector is should be small in size with single endonucleases site.
- But natural occurring rarely passes this characteristic.

COSMID:

- Cosmids are vectors posses the characteristic of both plasmid and bacteriophage.
- Cosmid can be constructed by adding a fragment of phase DNA to plasmid.
- A foreign DNA can be inserted into cosmid DNA.

BACs:

- Bacterial artificial chromosomes (BACs) is based on plasmid which is large than other plasmid used as cloning vector.