Biotechnology: Principles and Processes

INTRODUCTION

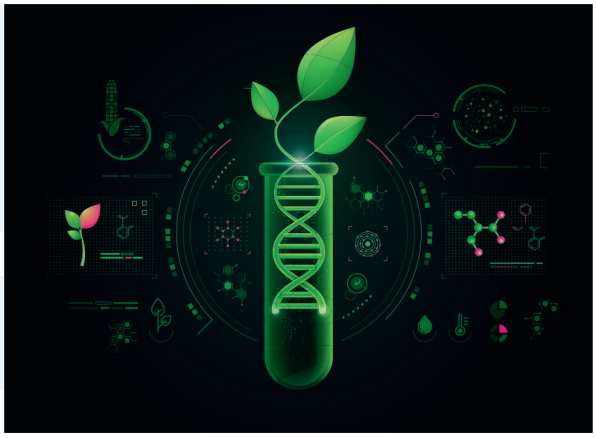
- The term biotechnology is of recent origin, but the discipline is very old. Humans had started using microorganisms as early as 5000 BC for making wine, vinegar, etc. Some of these processes have become so common in our homes that we use them in our daily works.
- Biotechnology is derived from two words — biology and technology. It is a field in which the biological agents and their components have been extracted and used for the generation of useful products. It is a vast technique which has improved and enlarged the scope of modifying organisms and their products for the humans and animals.
- **The European Federation of Biotechnology** has given a definition of biotechnology that takes into view the traditional as well as modern molecular biotechnology. The definition given by EFB is **"The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services".**
- Biotechnology is multi-disciplinary and uses biology, chemistry, biochemistry, genetics, molecular biology, immunology, cell tissue culture and physiology for the process of biotechnology.

PRINCIPLES OF BIOTECHNOLOGY

- Genetic engineering and Bioprocessing are the two core techniques that have led to the development of biotechnology.
  - **Genetic engineering**
  - **Bioprocessing**

Definition

Biotechnology: The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services.

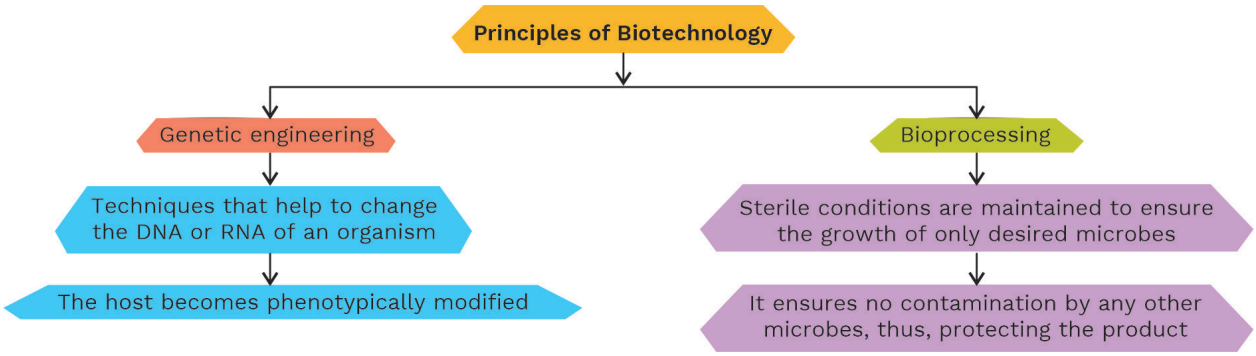


"The rewards for biotechnology are tremendous – to solve disease, eliminate poverty, age gracefully."

George M. Church

Previous Year's Question

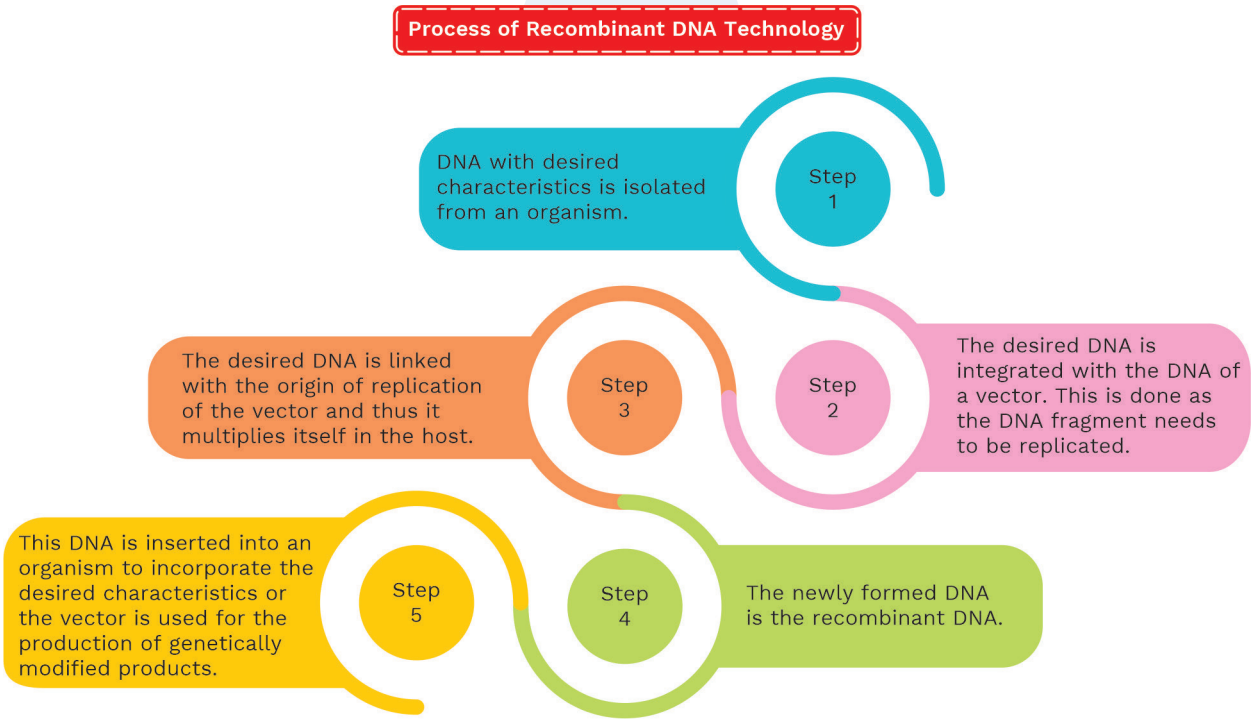
Introduction of foreign genes for improving genotype is
(1) tissue culture
(2) vernalization
(3) genetic engineering
(4) biotechnology



Definition

Recombinant DNA or rDNA: DNA molecule ligated into a vector and then inserted into a host organism to produce new genetic combinations.

PROCESS OF RECOMBINANT DNA TECHNOLOGY



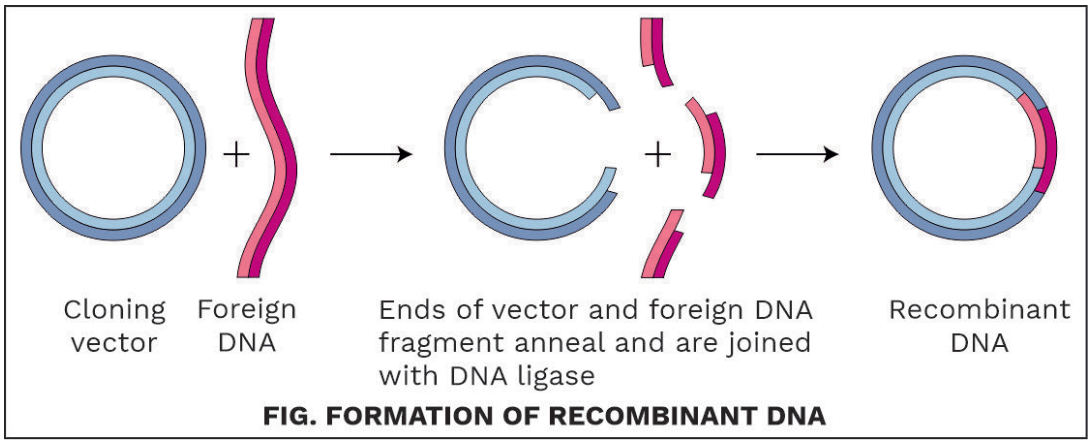


FIG. FORMATION OF RECOMBINANT DNA

CONSTRUCTION OF FIRST ARTIFICIAL RECOMBINANT DNA MOLECULE

- Plasmids are **extra-chromosomal** material present in the bacteria. It can be physically separated and can replicate independently of the bacterial chromosome. It provides **resistance** to the bacteria **against certain antibiotics.**
- First Artificial Recombinant DNA Molecule was constructed by **Stanley Cohen and Herbert Boyer in 1972.**
- Stanley Cohen and Herbert Boyer in 1972 isolated antibiotic resistant gene by cutting a fragment of DNA from a plasmid. This antibiotic resistant gene fragment was then integrated with the plasmid DNA.
- They cut the gene of antibiotic resistance from a plasmid with the help of a restriction enzyme and joined it to the plasmid of *Salmonella typhimurium* with the help of DNA ligase.
- This newly formed DNA having fragments of antibiotic resistance is known as the recombinant DNA.
- The plasmid of *Salmonella typhimurium* with the gene of antibiotic resistance acts as a vector and is transferred into the bacterium *Escherichia coli*.

Definition

Plasmid: Autonomously replicating extra-chromosomal material present in the bacteria that provides antibiotic resistance to the bacteria.

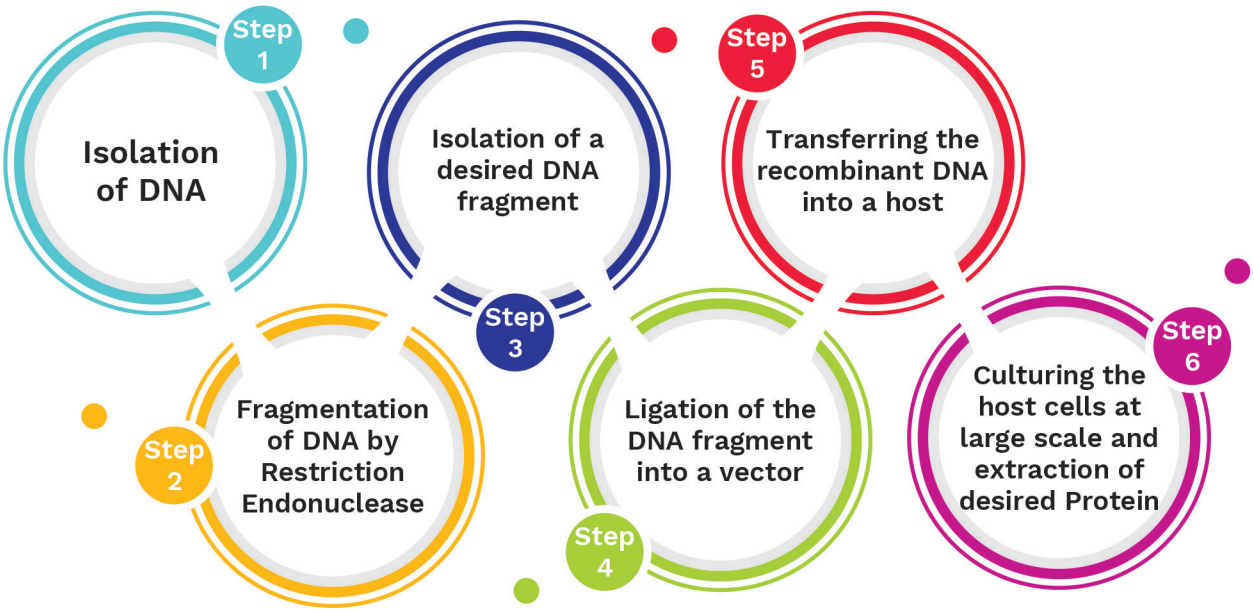
Previous Year's Question

Genetic engineering is possible, because

(1) we can cut DNA at specific sites by endonucleases like DNase
(2) restriction endonucleases purified from bacteria can be used in-vitro
(3) the phenomenon of transduction in bacteria is well understood
(4) we can see DNA by electron microscope.

- The plasmid was replicated in the bacteria *E. coli* with the help of the enzyme DNA polymerase and made multiple copies of the recombinant DNA.

STEPS INVOLVED IN THE PROCESS OF RECOMBINANT DNA TECHNOLOGY



Isolation of the DNA

- DNA is the genetic material of many organisms. In some organisms, it is the RNA.
- In the process of biotechnology, the fragment of DNA with the desired characteristics is first to be isolated from the source cell. The cell could be a plant, animal, bacterial or fungal cell.
- The DNA needs to be separated from the cell and the other macromolecules and then purified.

Keywords

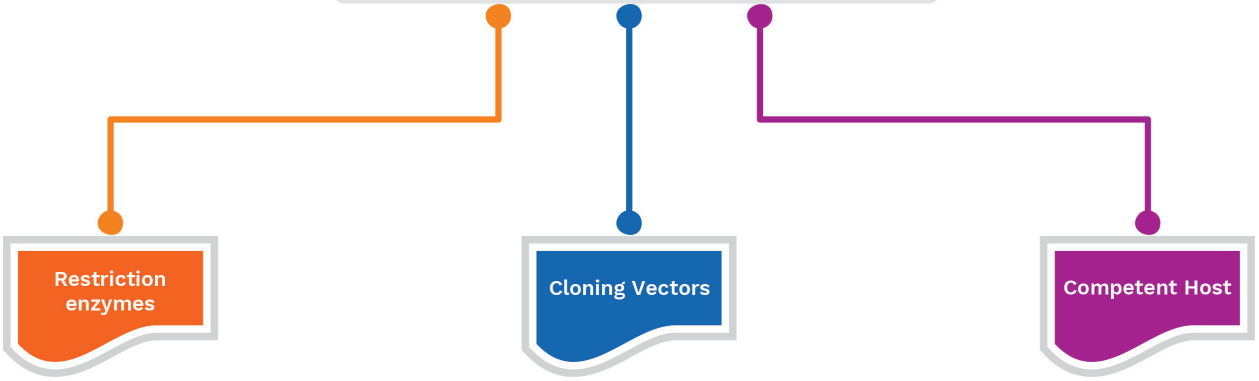
- Genetic Engineering
- Bioprocessing
- European Federation of Biotechnology
- Recombinant DNA
- Plasmid

- To cut the cell we need to lyse it with certain enzymes depending upon the type of cell from which the DNA needs to be isolated.
  - **Bacterial Cell-Lysozyme**
  - **Fungal Cell-Chitinase**
  - **Plant Cell-Cellulase**
- The DNA removed from the cell now need to separated from RNA, proteins and other macromolecules.
- RNA is removed by treating with ribonuclease while proteins are removed by treating with proteases.
- Chilled ethanol is now added to the soluble DNA and purified DNA precipitates out.
- The DNA is now removed by **spooling.**

FIG. SPOOLING OF DNA

Definition

Spooling: The precipitated DNA molecules is swirled with the glass rod or a spooling stick and separated from the solution.



Restriction Enzymes

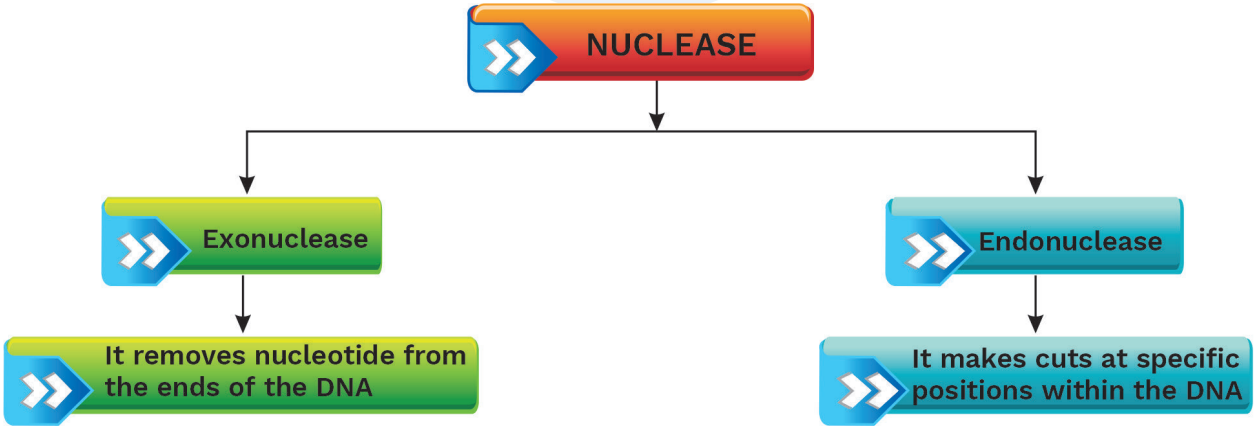
- **Steward Linn** and **Werner Arber** isolated two enzymes that restricted the growth of bacteriophage in *E.coli.*
- One restricted the growth of the bacteriophage by adding a methyl group to the DNA while the other cut the

DNA. The enzyme which cut the DNA is known as restriction enzyme. These enzymes are placed under the category of **Nucleases.**

- Restriction enzymes are of four broad categories
  - **Type I-**These enzymes cleave at random sites far away from the restriction sites. Thus they are not used in the recombinant technology.
  - **Type II-**These enzymes cut DNA at specific sites, close to or within their recognition sequences. They are used in biotechnology. Many of these restriction enzymes have been identified from a large number of bacterial species.

Previous Year's Question

Which of the following is not correctly matched for the organism and its cell wall degrading enzyme?
(1) Algae - Methylase
(2) Fungi - Chitinase
(3) Bacteria - Lysozyme
(4) Plant cells - Cellulase



  - **Type III-**These enzymes also cleave at sites a short distance from a recognition site far away from the restriction sites.
  - **Type IV-**These enzymes cleave only methylated DNA.
  - **Type V-**These enzymes use guide RNAs to target specific non-palindromic sequences present on invading organisms.

Rack your Brain

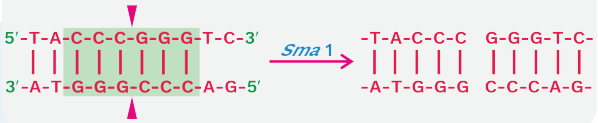
Restriction endonuclease is obtained from _____.

- **Restriction enzymes** recognize specific locations in the DNA strand where it makes the cut. The sequence at which the DNA strands recognize the specific site for cutting is known at the recognition sequence.
- These recognition sequences are usually palindromic sequences.
- **Palindromic sequences** are those sequences in which the sequence is read same in the forward and backward directions. For example, MALAYALAM
- In the DNA strand, sequence of the nucleotides when read from 5′ to 3′ in one strand and 3′ to 5′ in the other strand read the same.

5′—GAATTC—3′
3′—CTTAAG—5′

Gray Matter Alert!!!

Blunt ends or non-cohesive ends: A straight cut by the restriction enzymes generates strands that terminate in a base pair. It contains no unpaired DNA strand fleeting at the end of DNA.
These blunt ends are not desirable in biotechnology as the yield is lower with blunt ends.



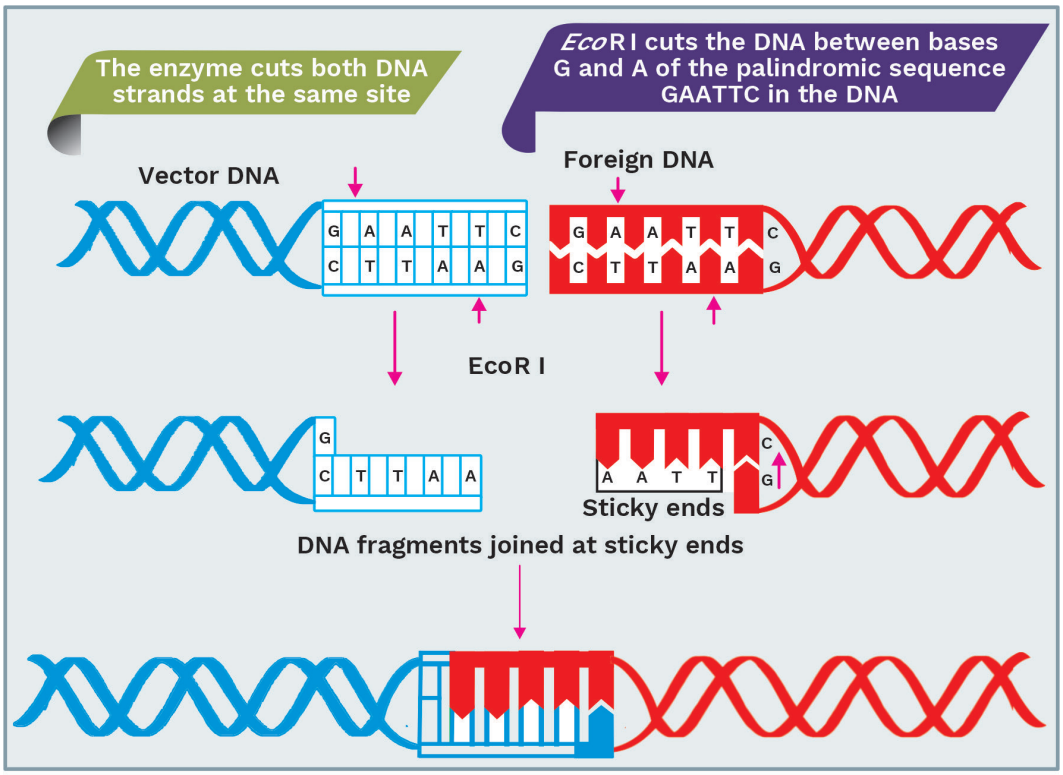


FIG. STEPS IN FORMATION OF RECOMBINANT DNA BY ACTION OF EcoR I (RESTRICTION ENDONCULEASE ENZYME)

- The restriction enzyme cuts the strand of DNA a little away from the center of the strand but between the same nucleotide sequences.
- This results in single stranded unpaired bases at the cut ends. These are known as **sticky ends** or **cohesive ends.**
- These sticky ends form hydrogen bonds with their complementary cut parts. When the DNA having the desired gene and the plasmid DNA are cut by the same restriction enzyme then the fragments will have the same sticky ends.

Previous Year's Question

There is a restriction endonuclease called EcoRI. What does "co" part in it stand for?
(1) colon
(2) coelom
(3) coenzyme
(4) *coli*

Nomenclature of the Enzymes

- The restriction enzymes isolated from the bacteria are named according to the source from which they have been isolated. The rules of nomenclature are—

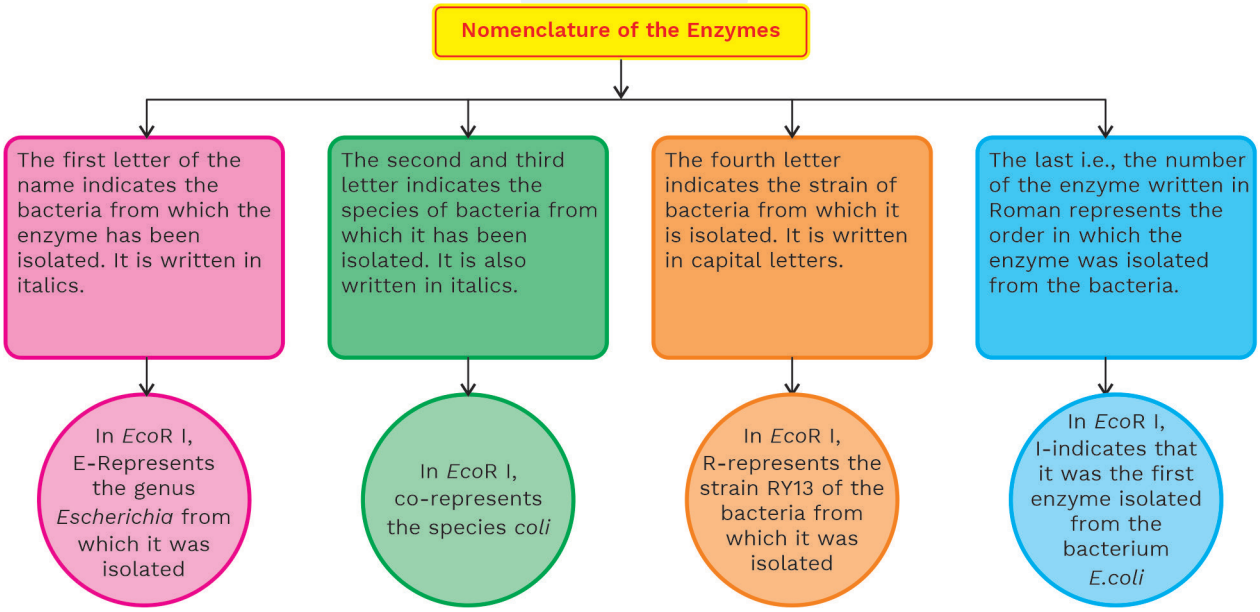


Table. Sources of Different Restriction Enzymes

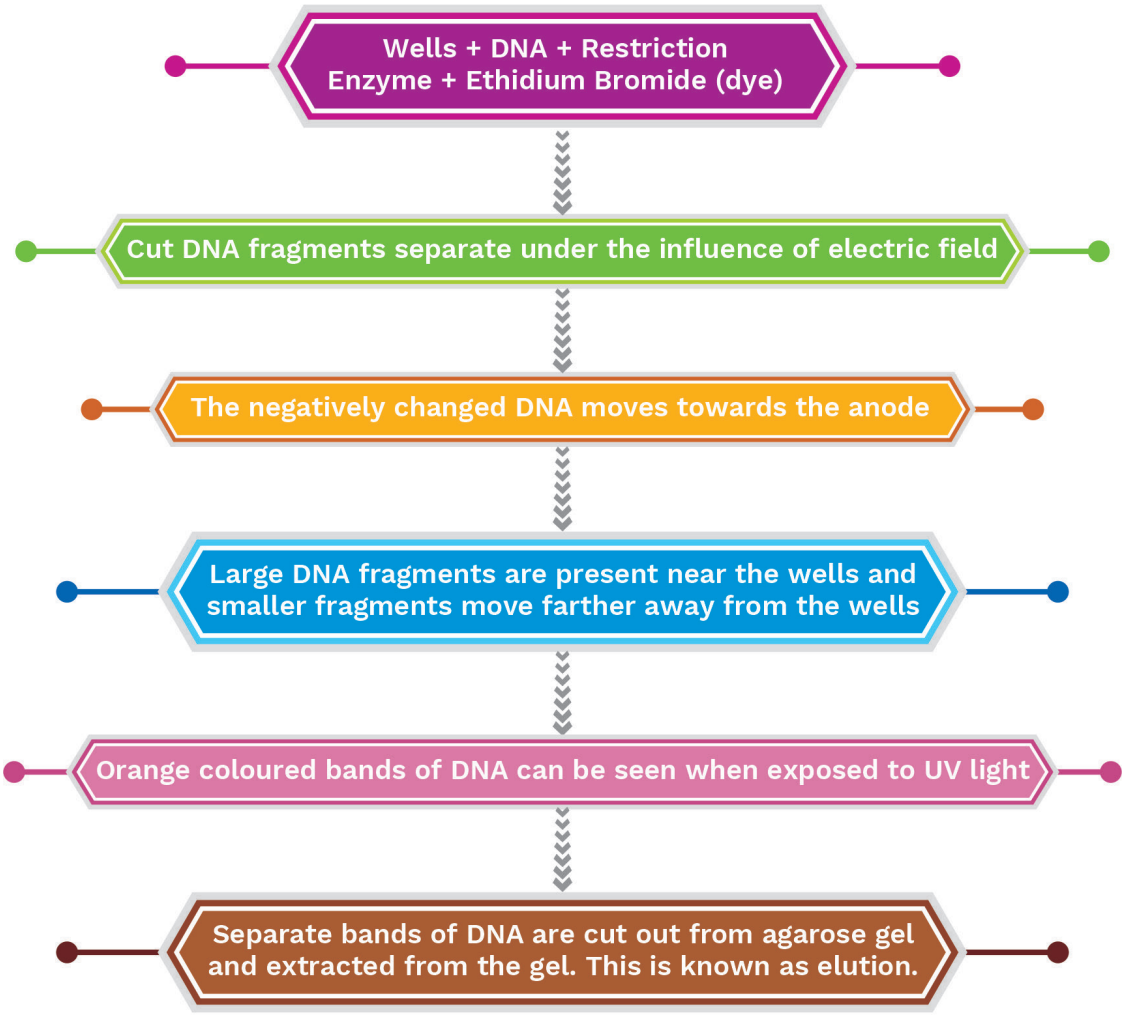
Enzyme Name	Genus	Species	Strain
BamH I	*Bacillus*	*amyloliquefaciens*	H
Sal I	*Streptomyces*	*albus*	-
Pst I	*Providencia*	*stuartii*	-
Pvu I	*Proteus*	*vulgaris*	-
Cla I	*Caryophanon*	*latum*	-
*Hin*D III	*Haemophilus*	*influenzae*	DSM 11121

Gel Electrophoresis

- The process of separation of DNA, RNA or protein fragments under the presence of electric field is known as gel electrophoresis. The DNA segments need to be separated into different fragments according to their sizes and then the desired fragment needs to be separated.
- **Materials required:** Desired DNA fragment to be cut, agarose gel, gel plate, ethidium bromide
- **Principle involved: DNA** being **negatively charged** moves towards the positive electrode i.e. anode under the influence of electric field.
- **Procedure:** Electrophoresis is performed in a gel matrix.

Rack your Brain

What is the principle on which gel electrophoresis works?



Steps of Gel Electrophoresis

Definition

Elution: The separated DNA fragments are cut from the agarose gel and extracted from it.

Keywords

- Nuclease
- Recognition sequence
- Palindrome sequence
- Gel electrophoresis
- Elution

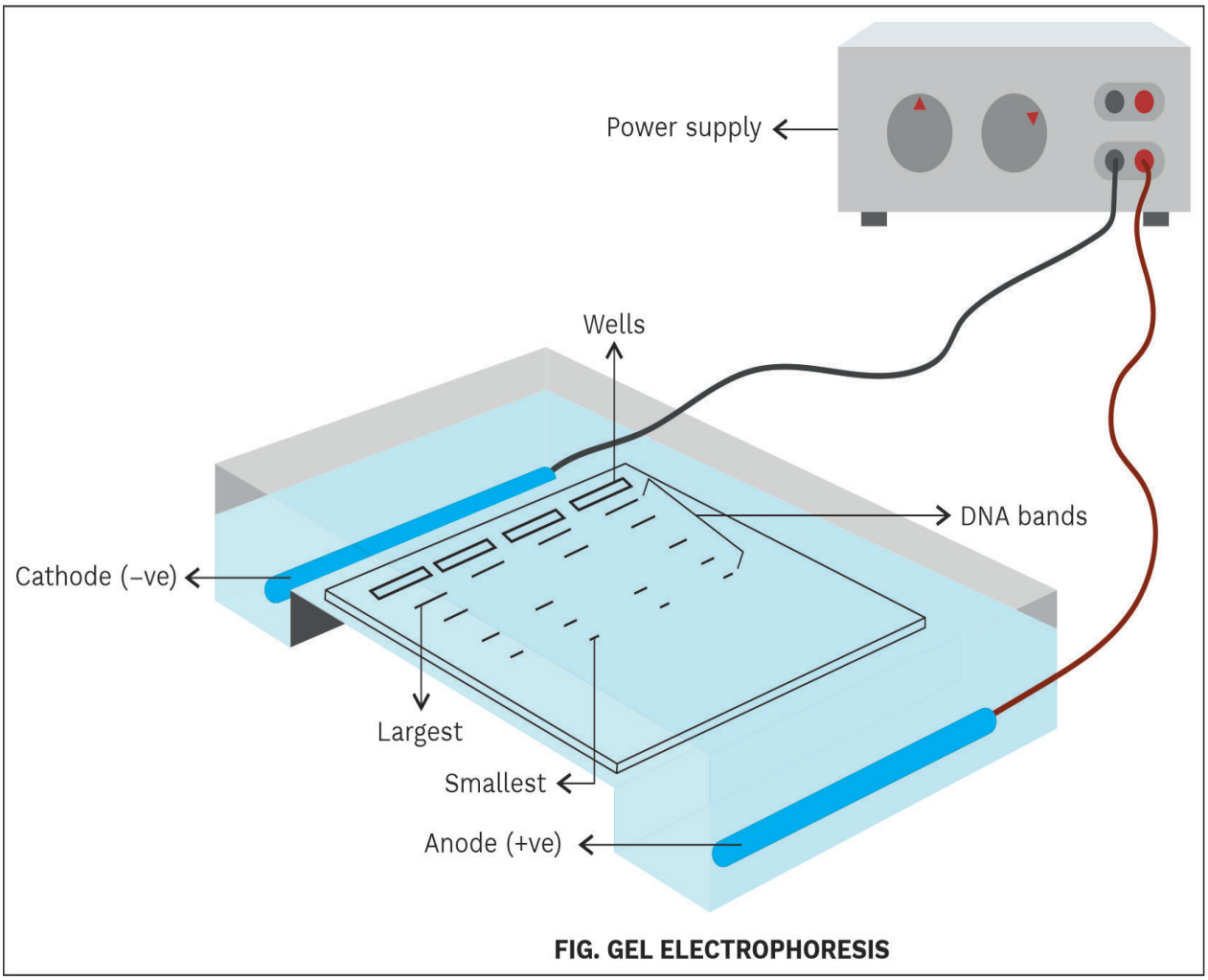


FIG. GEL ELECTROPHORESIS

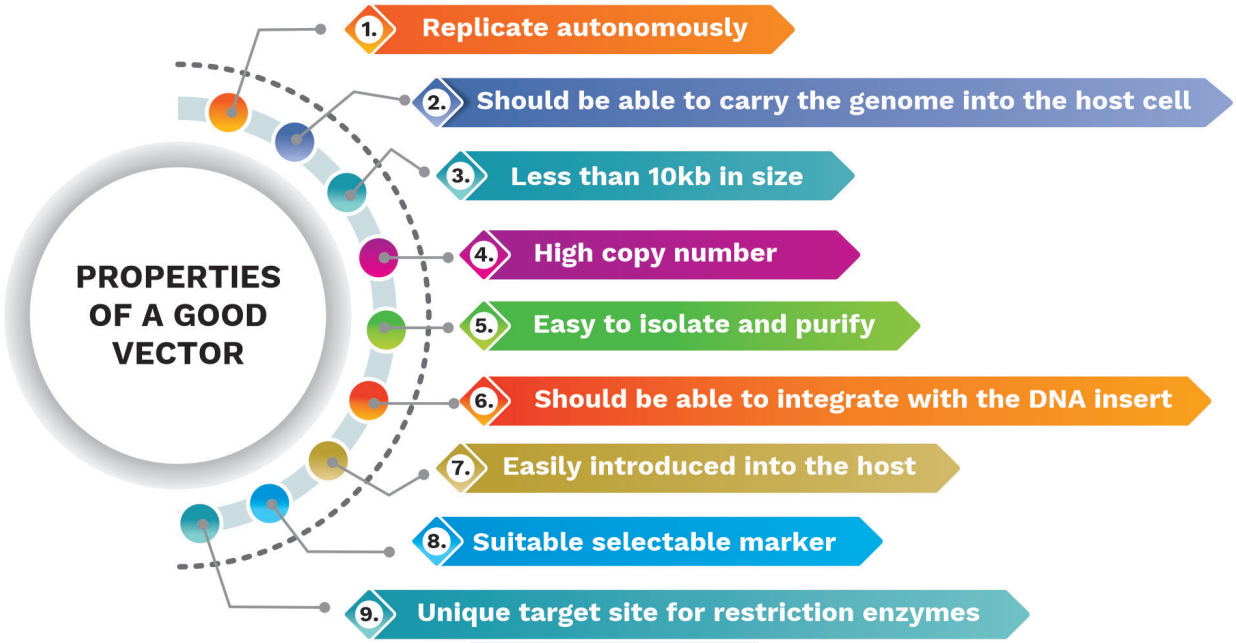
Cloning Vectors or Cloning Vehicle

- Cloning vectors are structures that help in making multiple copies of the desired DNA fragments.
- These vectors can replicate within the host bacterial cell. Some of them are present naturally in the bacterial cell, while in others they can be introduced artificially.

Previous Year's Question

Agarose extracted from sea weeds is used in

(1) spectrophotometry
(2) tissue culture
(3) PCR
(4) gel electrophoresis



CHARACTERISTIC FEATURES OF CLONING VEHICLES (VECTORS)

- A vector is a DNA molecule that has the ability to replicate inside the host cell.
- A vector can be inserted with the foreign desired DNA fragments for cloning.
- To act as a vector and to facilitate cloning a vector must have the following characteristics–
  - **Origin of Replication or ori**
    - It is a set of sequence which is present on the vector from which the replication of the DNA starts.
    - The desired foreign DNA which is isolated from the organism needs to be integrated with the vector at the origin of replication or near to the origin of replication of a vector, then it can replicate the desired DNA fragment during its own replication. It also controls the **copy number.**

Rack your Brain

Name the technique used for separating the DNA fragments in a laboratory?

Definition

Copy Number: It is the average number of copies of the plasmids made per host cell.

- **Restriction Sites or Recognition Sites**
  - Each vector should have only one recognition site of a particular enzyme.
  - If it has more than one recognition site for a particular enzyme then managing the vector DNA would be difficult as it would be cut at different positions and thus integration of the new DNA would be difficult.
- **Selectable Markers**
  - Vectors should have selectable marker which helps in identifying and eliminating the non-recombinant or non-transformants from the recombinants or the transformants.
  - Transformation is the process through which a piece of DNA is introduced into the bacteria so that it can be integrated with the vector present inside the bacteria.
  - Usually **genes encoding resistance for antibiotics** like ampicillin, chloramphenicol, tetracycline or kanamycin are considered as useful selectable markers.
- **Molecule Weight –** Low
- **Sites for different restriction enzymes –** Several sites for a large number of restriction enzymes.

Definition

Transformation: The uptake of DNA fragment by the cell and integration into its own chromosomal DNA and expressing the trait of the incorporated DNA.

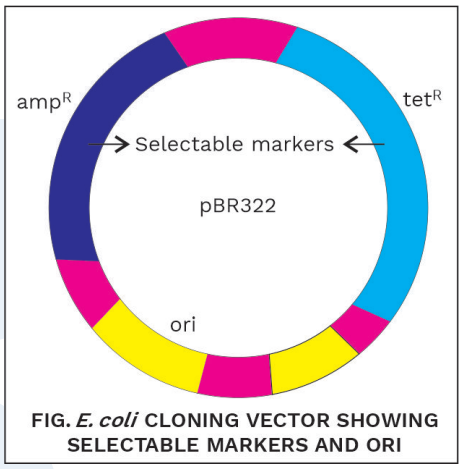


FIG. *E. coli* CLONING VECTOR SHOWING SELECTABLE MARKERS AND ORI

Previous Year's Question

A selectable marker is used to
(1) help in eliminating the non-transformants, so that the transformants can be regenerated
(2) identify the gene for a desired trait in an alien organism
(3) select a suitable vector for transformation in a specific crop
(4) mark a gene on a chromosome for isolation using restriction enzyme

TYPES OF VECTORS

Plasmids

- They are the most commonly used cloning vectors. They are circular double-stranded DNA molecule that occurs as an extra-chromosomal material in the bacteria. Naturally they are self replicating usually one or two copies of the plasmid are present in a cell. The size of plasmid may range from 1 to 200 bps.
- Plasmids can be of two types- **single copy plasmid** or **multiple copy plasmid.**
  - Single copy plasmid maintains one plasmid per host while multiple copy plasmids can present in large numbers inside the host.
- Plasmid vector is isolated from the bacterial cell.
- Foreign DNA carrying the desired gene for a particular characteristic is then integrated with the DNA of the vector and joined together.
- The newly formed recombinant DNA is now inserted into the bacterial cell for cloning.
- One of the earliest vectors that has been constructed artificially is the pBR322. p stands for plasmid, B is for Boliver, R is for Rodriquez, initials of the scientist who developed pBR322. The numerical 322 distinguishes this plasmid from the other plasma that developed in the same lab.
- pBR322 is the most popular and widely used plasmid which has 4361 base pairs in length. Its entire sequence is known.
- It has two selectable markers tetracycline and ampicillin resistant genes and many different recognition sites for restriction enzymes. The

Previous Year's Question

The plasmid pBR322 used in biotechnology is
(1) *Yeast*
(2) M13 phase
(3) parasite
(4) cloning vehicle

Rack your Brain

Name the type of host cells suitable for the introduction of an alien DNA.

Previous Year's Question

Which of the following is not a feature of the plasmids?
(1) Transferable
(2) Single-stranded
(3) Independent replication
(4) Circular structure

presence of the restriction sites helps in easy selection for the recombinant pBR322.

- Insertion of the DNA fragment into the plasmid at the *Pst* I or *Pvu* I, places the DNA insert within the gene ampicillin and thus makes it non-functional.
- Bacterial cell containing such a recombinant pBR322 will be able to grow in the presence of tetracycline but will not grow in ampicillin.
- Similarly when DNA insert is placed in the restriction enzyme *Bam*H I and *Sal* I, the gene coding for tetracycline resistance becomes non-functional.
- This is a cumbersome process and requires multiple plating. To avoid this process which takes a lot of time we insertional inactivation is favoured which helps us to identify the transformants from the non-transformants easily by the inactivation of a gene by insertion of a DNA sequence within it.

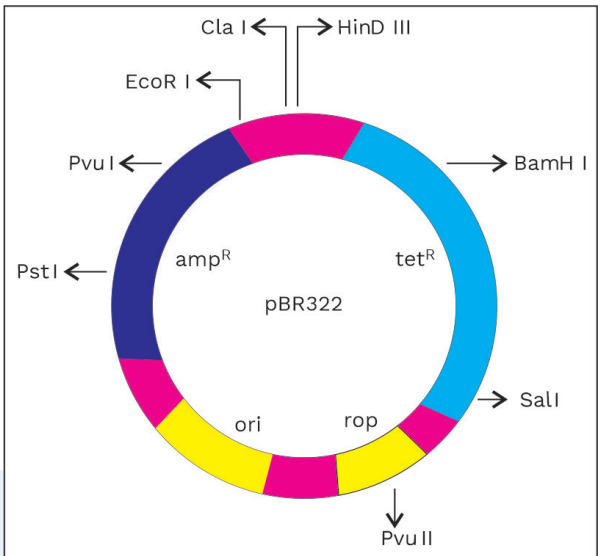


FIG. *E. coli* CLONING VECTOR SHOWING RESTRICTION SITES

Identification of Recombinants

- Once a recombinant DNA molecule has been introduced into the vector and the vector is introduced into the bacterial cell it is important to isolate the transformants from the non-transformants.
- This can be done by growing the bacterial cell on antibiotics and thus this helps us to identify the transformers and separate them from the non-transformers.
- Let's understand this with the example of pBR322.
- It may happen that the bacteria *E coli* may get inserted with a normal vector which does not have the recommend

Rack your Brain

What will happen if a foreign DNA is ligated at *Bam*H I site of PBR322 plasmid?

DNA inserted into it. So we need to select those bacteria which have the recombinant vector inserted into it.

- This can be done with the help of a procedure called replica plating.
- After trying to transform pBR322 plasmid with the desired DNA, the host cell is first grown on agarose gel containing antibiotic ampicillin.
- Here we assume in this case that the recombinant DNA has ligated with the tetracycline resistance gene.
- Colonies will develop on the single plate. In the plate both the bacterial cells having the vectors with the recombinant DNA and the ones not having the recombinant DNA would multiply.
- The role of the recombinant plasmid is to help the host cell to multiply in the presence of antibiotic which otherwise would not be able to do.
- A petri plate containing solid media with antibiotic tetracycline is kept carefully under aseptic conditions. To this, a circular piece of velvet is aligned and pressed onto the colony containing ampicillin plate (master plate).
- With the same alignment it is pressed onto the tetracycline plate.
- Overnight, only colonies not containing the insert will grow. No colonies which have the insert at tetracycline position will grow.
- The colonies which have the insert can easily be selected by comparing the two plates.
- The above described process is a time taking one and so another process of insertional inactivation is used.

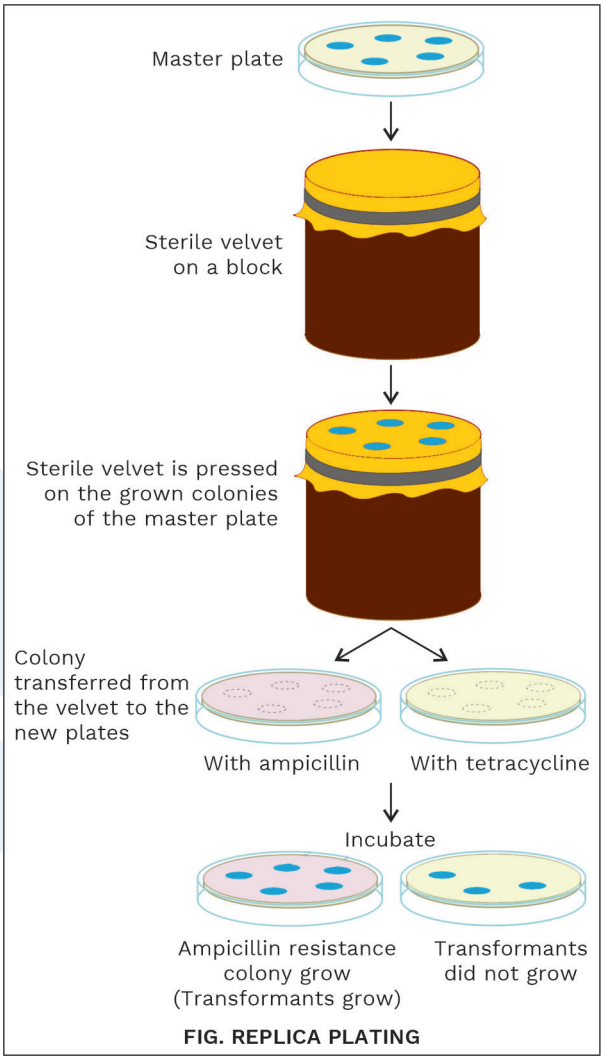


FIG. REPLICA PLATING

Previous Year's Question

A gene whose expression helps to identify transformed cell is known as

(1) vector
(2) plasmid
(3) structural gene
(4) selectable marker

- In this process a recombinant DNA is inserted into the coding sequence of β galactosidase. This bring about **insertional inactivation.**
- If the plasmid in the bacteria does not have an insert the presence of chromogenic substrate gives **blue coloured colonies.**
- If the plasmid in the bacteria have an insert then the colonies **do not produce any colour** and these are the colonies that are recombinant colonies.

Previous Year's Question

Selectable marker is used to
(1) eliminate the non-transformants
(2) identify the gene for a desired trait
(3) select a suitable vector for transformation in a specific crop
(4) mark a gene on a chromosome for isolation using restriction enzyme

Bacteriophage

- Bacteriophages are viruses that attack bacteria.
- They follow lytic or lysogenic cycle. In the lysogenic cycle, the phage DNA integrates with the bacterial chromosome and multiply.
- The two bacteriophages that have been used are lambda and M13 phage
  - **Lambda phage-**The phage has 48,502 base pairs and it is double-stranded, linear DNA and has single-stranded protruding cohesive ends of 12 bases.
    - The genome is linear in the phage head but in the *E.coli,* the two cohesive ends anneal to form a circular molecule. The sealed cohesive sites are known as COS sites.
  - **M13 phage -** Filamentous phage having a linear DNA which converts into a double-stranded circular intermediate form within the host referred to as the Replicative Form (RF).
    - **Advantage** over plasmid-More efficient.

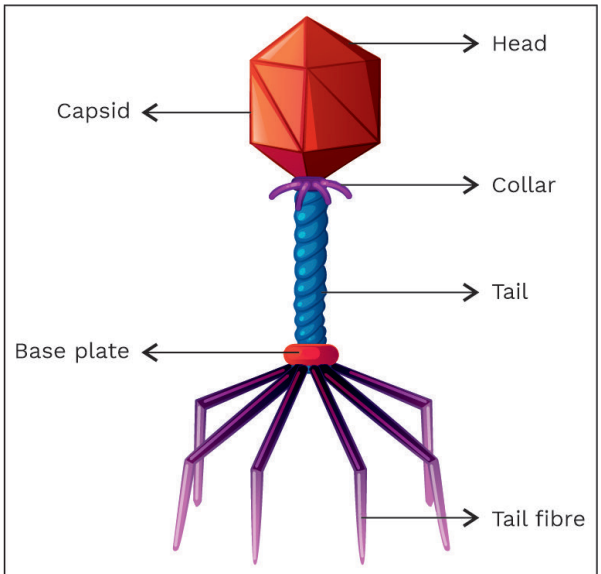


FIG. BACTERIOPHAGE

- Easy to clone large amount of DNA.
- Easy to screen a large number of recombinant phage.

Cosmids

- It is a combination of **plasmids** and 200 base pairs of lambda phage containing the COS site of the **lambda phage.**
- It also has a replication origin, unique restriction sites, a selectable marker from the plasmid.
- Cosmids can accommodate 40-45 kb of DNA insert in it.

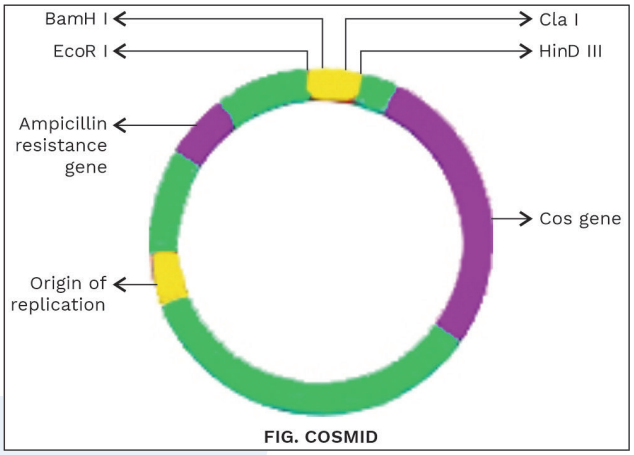


FIG. COSMID

Shuttle vector

- They can replicate in two different species and so contain two origins of replication specific for each host.
- Some can grow in two different Prokaryotic species for example in *E coli* and *Streptomyces* and some in Eukaryotic species like *Yeast*.

Yeast Artificial Chromosome Vector (YAC)

- Linear vectors that behave as yeast chromosomes. It consists of a sequence for replication, one or two selectable markers, Telomeric sequences at both the ends that protect from exonuclease activity.
- It was used in **human genome project.**

Bacterial Artificial Chromosome Vectors (BAC)

- They have sequence of replication of origin. It controls the stability to maintain 1-2 copies of the vectors per cell.
- It can accommodate inserts up to 500 kb. It contains a selectable marker and

Previous Year's Question

Which vector can clone only a small fragment of DNA?

(1) Bacterial artificial chromosome
(2) Yeast artificial chromosome
(3) Plasmid
(4) Cosmid

cloning sites, and genes for replication and maintenance of the F plasmid.

- It was used in **human genome project.**
- **Phasmid-**They consist of linear lambda genome consisting of DNA replication.
- **Transposons-**First observed by Mc Clintock in maize. Units of DNA that can move from one DNA to another DNA and hence are mobile. They are also called as mobile genes or jumping genes.

Animal Vectors

- DNA fragments from animals are cloned in *E.coli* and then integrated with different viruses to be introduced into the host animal cell.
- The ability of viruses to adsorb to cells, introduce their DNA and replicate the DNA , have made them ideal to transfer foreign DNA into eukaryotic cells.
- Adenoviruses and Papillomavirus have been used to clone genes in mammals. Retroviral vectors are used for cloning genes in mammalian cells.
- **Retrovirus is disarmed** of its harmful DNA and then in its place the desired DNA fragment is inserted to be integrated with the host.

Plant Vectors

- Two plasmids namely Ti and Ri are present in *Agrobacterium tumefaciens* and *A. rhizogenes* respectively and are considered as natural genetic engineer for the plants.
- **Ti** is a **tumour inducing** plasmid while **Ri** is a **root inducing** plasmid.
- Ti plasmid is around 200kb size.

The process of infection by *A. tumefaciens* is because of the transfer

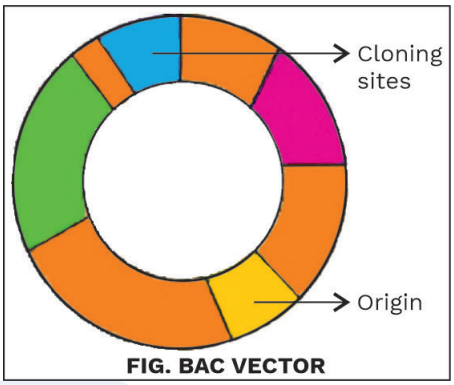


FIG. BAC VECTOR

Previous Year's Question

Which one of the following is commonly used in transfer of foreign DNA into crop plants?

(1) *Meloidogyne incognita*
(2) *Agrobacterium tumefaciens*
(3) *Penicillium expansum*
(4) *Trichoderma*

of a small part of Ti into the plant cell genome. The DNA sequence is known as T-DNA i.e. transferred DNA.

- *vir* region regulates the transfer of T-DNA into the plant cell.
- *A.tumefaciens* produces nitrogenous compounds called opines like octopines or nopaline which are used by the plasmid as their carbon and nitrogen source. While *A. rhizogene* produces either agropine or mannopine. Opine catabolism region produces enzymes required for the utilization of opines by Agrobacterium.
- Both the plasmids have the genes for Indole acetic acid and cytokinin which help in rapid division of the cells.

COMPETENT HOST

Vector Mediated Gene Transfer

- The recombinant DNA now needs to be incorporated into a cell which can be a bacteria, plant or animal cell.
- DNA is hydrophilic and it is not possible for it to pass through the membranes.
- The bacterial cell is treated first with **high concentration** of **calcium** ions to increase the efficiency of the membrane to intake DNA.
- The cells are then incubated on **ice bath** and then given a **heat treatment** at 42°C and again put on ice bath.
- This helps the bacteria in taking up the DNA.

Direct Gene Transfer

- Introduction of DNA into the plant cell or animal cell directly without the use of a biological agent is known as direct gene transfer. The spontaneous uptake of DNA by the plant or animal cell is less and thus there are different ways to increase the uptake.

Rack your Brain

Why DNA cannot pass through the cell membrane?

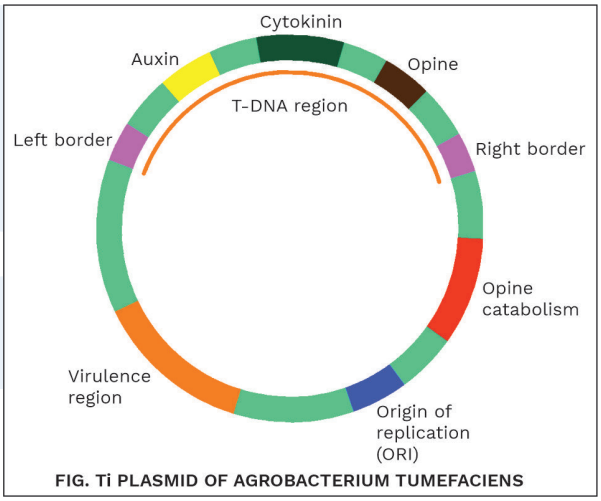


FIG. Ti PLASMID OF AGROBACTERIUM TUMEFACIENS

Rack your Brain

Why is it important to make bacterial cell competent?

- The various method of direct gene transfer is–
  - Chemical method
  - Electroporation
  - Biolistic
  - Microinjection
  - Macroinjection
  - Lipofection
- **Chemical Method**
  - **Polyethylene glycol, polyvinyl alcohol** is used to increase the intake of DNA by the plant cell.
  - The plant protoplast is placed in a medium rich in Mg^{2+}. To this, the plasmid DNA with the desired gene is added.
  - After this, Polyethylene glycol is added and pH is maintained at 8.
  - The protoplast is given a five minute heat treatment and then placed on ice before the addition of DNA.
  - This increases the frequency of transformation.
  - After some period of incubation the polyethylene glycol is reduced and calcium ions are increased.
  - This helps in increasing the transformation frequency.
- **Electroporation**
  - The cells are exposed to electric impulse which induces transient pores in the plasmalemma that helps in the intake of the DNA.
  - Plant protoplast is suspended in an ionic medium containing the recombinant plasmid. The mixture is subjected to voltage and thus help in the uptake of DNA.

Keywords

- Ori
- Selectable marker
- Insertional Inactivation
- Ti Plasmid
- Microinjection
- Biolistics

Rack your Brain

Name the natural genetic engineer of plants.

Previous Year's Question

Electroporation procedure involves
(1) fast passage of food through sieve pores in phloem elements with the help of electric stimulation
(2) opening of stomatal pores during night by artificial light
(3) making transient pores in the cell membrane to introduce gene contruct
(4) purification of saline water with the help of a membrane system

- The following two ways of electroporation can be used–
- Low voltage long pulse-For this way 300-400Vcm^{-1} is used.
- High Voltage short pulse-For this way 1000-1500Vcm^{-1} is used.
- Electroporation has been used in intact plant cells too.
- Electroporation has been used in Tobacco, *Petunia*, Maize, Rice and Wheat.

- **Biolistics or Gene gun**
  - 1-2 mm **Tungsten** or **gold** particle coated with DNA is used for transformation.
  - These particles are accelerated at **high velocities** and thus this enables it to enter into the plant cell.
- **Lipofection**
  - The desired DNA is introduced into the cell with the help of liposomes. This method can be used for animals as well as the plants.
- **Microinjection**
  - The DNA solution is injected directly into the cell with the help of glass micropipettes.
  - Protoplasts should be used as it does not interfere with the use of microinjection but if an intact cell is used then cell wall interferes with the process of microinjection.
  - The protoplast is held on a glass slide coated with polylysine or held under the suction by a micropipette and then the DNA is injected.
  - It is a time taking process.

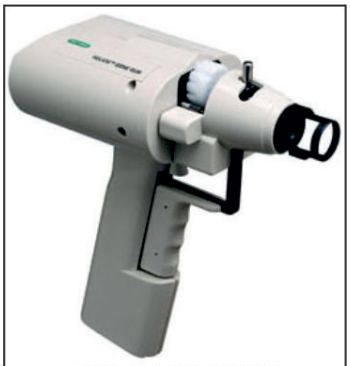


FIG. GENE GUN

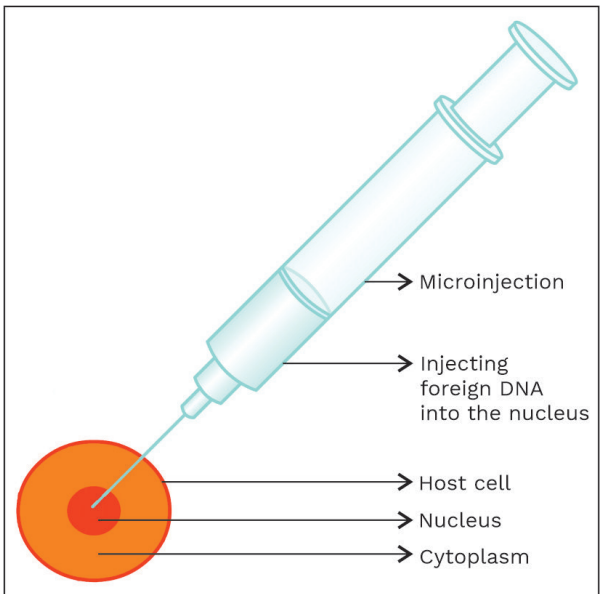


FIG. MICROINJECTION

- **Macroinjection**
  - In this process, plasmid DNA is injected into the lumen of the inflorescence and using a hypodermic syringe.

OBTAINING THE FOREIGN GENE PRODUCT

- The recombinant DNA is inserted into the bacterial cell, plant cell or animal cell. Then the foreign DNA is multiplied .
- The recombinant DNA should produce the desired protein.
- After the cloning of the gene, optimum conditions should be maintained for the production of the proteins.
- If a protein-encoding gene is expressed in a heterologous host then it is known as **recombinant protein.**
- The cells can be grown at a small scale in the laboratory .But in this case, the amount of proteins produced will be less.
- To ensure the production of large scale of proteins, large vessels known as bioreactors are used.
  - **Bioreactors or fermenters**
    - Large vessels in which the raw materials are biologically converted into the products by the microbes, plant and animal cells or enzymes are known as bioreactors.
    - Bioreactors can be aerated, stirred for mixing of the medium. They can be made contamination-free and replacement of used medium takes place.
    - Usually the most commonly used bioreactor is the stirring type. They are of two types-

Definition

Macroinjection: The process of injecting the plasmid DNA into the lumen of the inflorescence with a hypodermic syringe.

Previous Year's Question

Biolistics (gene gun) is suitable for
(1) disarming pathogen vectors
(2) transformation of plant cells
(3) constructing recombinant DNA by joining with vectors
(4) DNA fingerprinting

Definition

Recombinant Protein: If a protein encoding gene is expressed in a heterologous host then it is known as recombinant protein.

Simple stirred tank bioreactor
Sparged stirred tank bioreactor

- **Simple stirred-tank bioreactor-** These are simple type and consists of a stirrer which help in mixing and even distribution of oxygen throughout the reactor.
- It is usually cylinderical or has a curved base to help in mixing the contents.
- Arrangements are provided to maintain temperature, pH, foam and delivery of oxygen to the vessel.
- **Sparged stirred-tank bioreactor-** In it, air is supplied in the form of **air bubbles.** The surface area for oxygen transfer is increased.
- Fermentation of the raw materials is carried out in the bioreactors. Two types of fermentation can take place namely **batch fermentation** and **continuous fermentation.**
- In batch fermentation nutrients, raw materials are put in a closed chamber and not changed once the fermentation starts.
- In continuous fermentation, the nutrients and raw material are added continuously and products are removed as they are made.

DOWN STREAMING

- After the product has been made in the bioreactor, it needs to be processed before it is marketed.

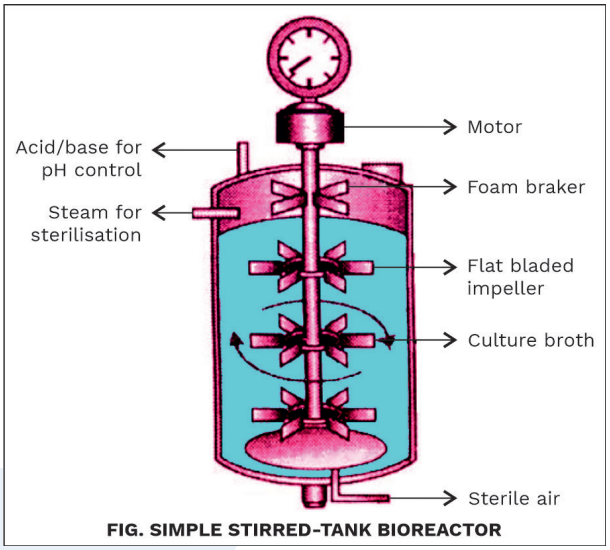


FIG. SIMPLE STIRRED-TANK BIOREACTOR

Previous Year's Question

Stirred-tank bioreactors have been designed for
(1) purification of product
(2) addition of preservatives to the product
(3) availability of oxygen throughout the process
(4) ensuring anaerobic conditions in the culture vessel

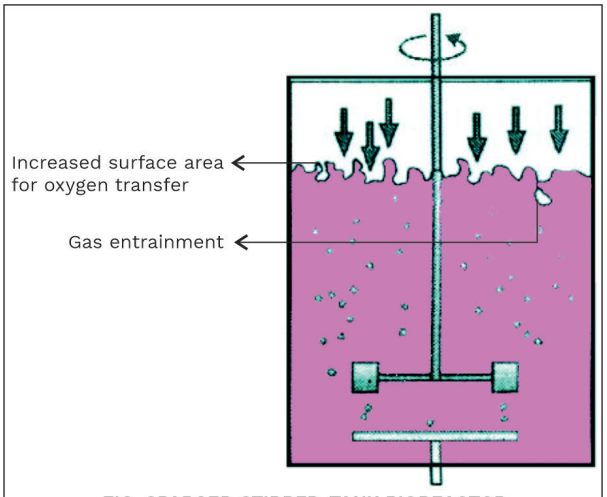


FIG. SPARGED STIRRED-TANK BIOREACTOR

- The process includes **separation** and **purification** of the product before it is marketed and it is together known as downstreaming.
- After down streaming process many clinical trials need to be done so that the product is checked before making it commercial.

Definition

Downstream Processing: Separation and purification of the product is known as downstream processing.

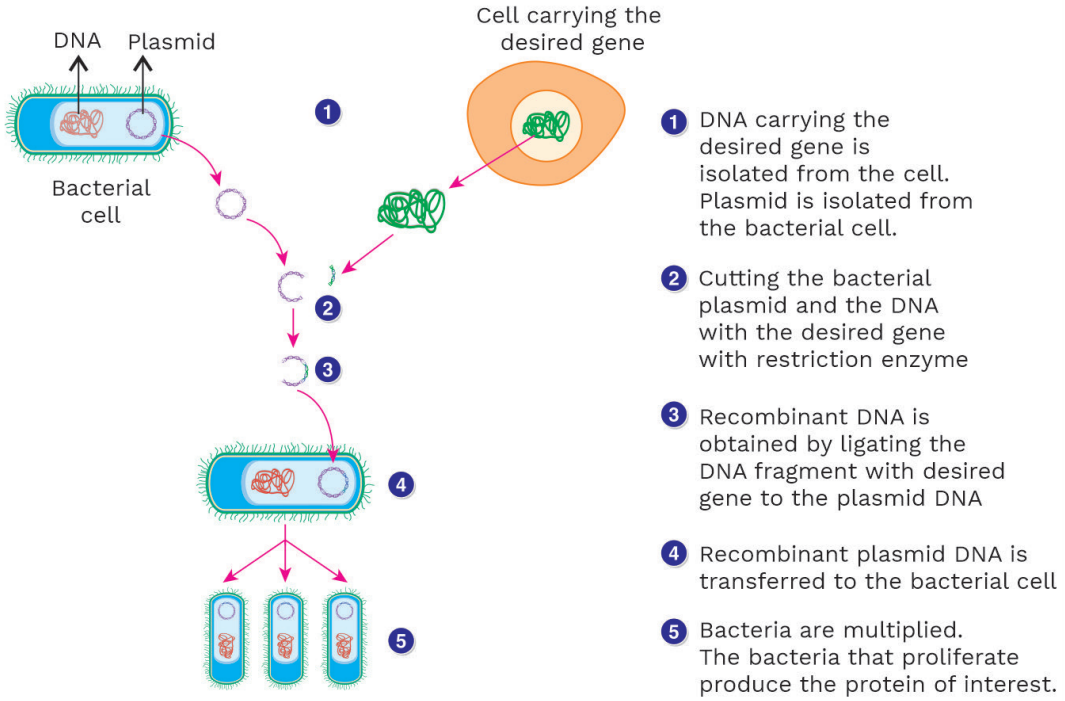


FIG. DIAGRAMMATIC REPRESENTATION OF RECOMBINANT DNA TECHNOLOGY

GENE AMPLIFICATION THROUGH POLYMERASE CHAIN REACTION

- The polymerase chain reaction (PCR) technique, developed by **Kary Mullis.** It generates upto billion copies of the desired DNA (or RNA) segment. The PCR process is completely.
- The PCR is carried out in vitro. It utilizes the following:
  - a DNA containing the desired segment of gene.

Previous Year's Question

PCR and restriction fragment length polymorphism are the methods for
(1) study of enzymes
(2) genetic transformation
(3) DNA sequencing
(4) genetic fingerprinting

- two nucleotide primers (about 20 bases long)
- the four deoxyribose nucleotide triphosphates, dTTP (Deoxythymidine triphosphate), dCTP (deoxycytidine triphosphate), dATP (deoxyadenosine triphosphate) and dGTP (deoxyguanosine triphosphate)
- a heat stable DNA polymerase, e.g., *Taq* (isolated from the bacterium ***Thermus aquaticus***)

Procedure of PCR

- It consists of the following steps-

Denaturation

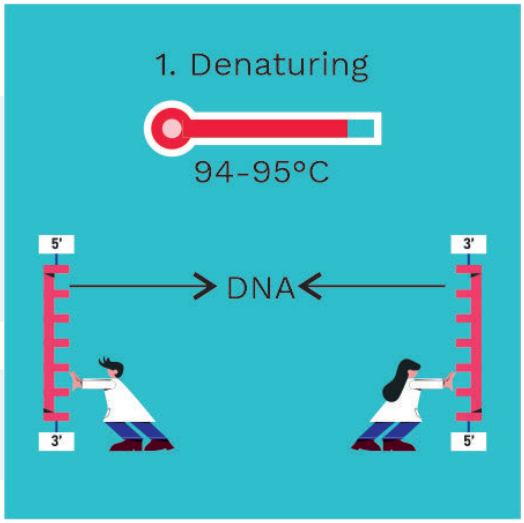
- The mixture containing the DNA is first heated to temperature between 90–98°C.
- This brings about DNA denaturation. The hydrogen bonds between the base pairs break and this leads to the separation of the DNA strands.
- Each single strand then acts as a template for DNA synthesis.
- The duration of this step in the first cycle of PCR is usually 2 min.

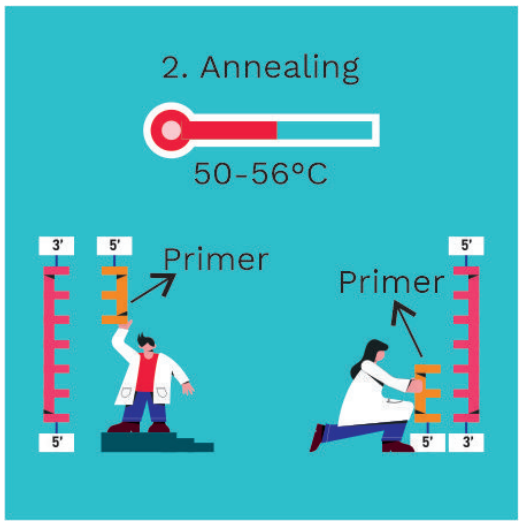
Annealing

- The mixture is now cooled to a temperature that permits annealing of the primer to the complementary sequences in the DNA.
- Two oligonucleotide primers anneal to each of the single-stranded template DNA.
- These sequences are located at the 3′ ends of the two strands of the desired segment. This step is called annealing.
- The primer concentration is kept very high relative to that of the template

Rack your Brain

What is the source of thermostable DNA polymerase?

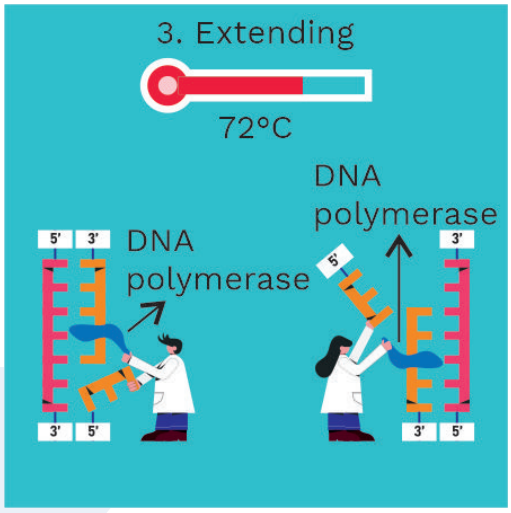


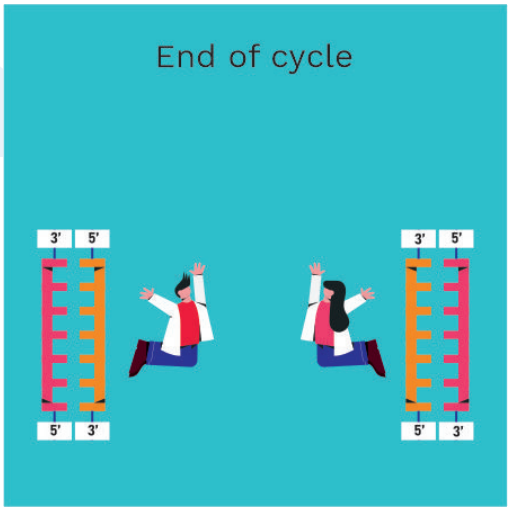


DNA, primer-template hybrid formation is greatly favoured.

Primer Extension

- The final step is extension.
- *Taq* DNA polymerase (of a thermophilic bacterium *Thermus aquaticus*) synthesizes the DNA region between the primers, using dNTPs (deoxyribose nucleoside triphosphates) and Mg^{2+}.
- DNA polymerase synthesizes the complementary strands utilizing 3′ OH of the primers.
- The primers are extended towards each other so the DNA segment lying between the two primers are copied.
- The duration of primer extension is usually 2 min at 72°C.
- The original template sequence will be copied during the second and all the other subsequent cycles.
- At each cycle, both new and old strands anneal to the primers and serve as templates for DNA synthesis. Thus at the end of each cycle, the number of copies of the desired segment becomes twice the number present at the end of the previous cycle.
- After PCR cycles, the amplified DNA segment is purified by gel electrophoresis and used for the desired purpose.





Rack your Brain

What is the advantage of PCR over the normal method of using plasmid for gene cloning?

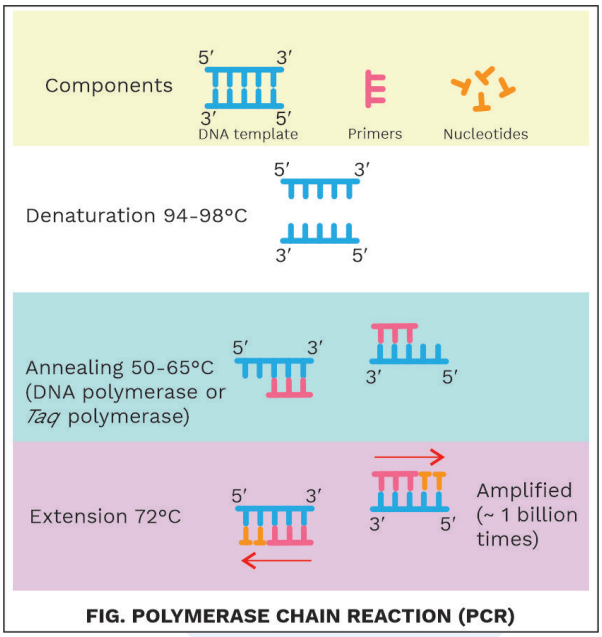
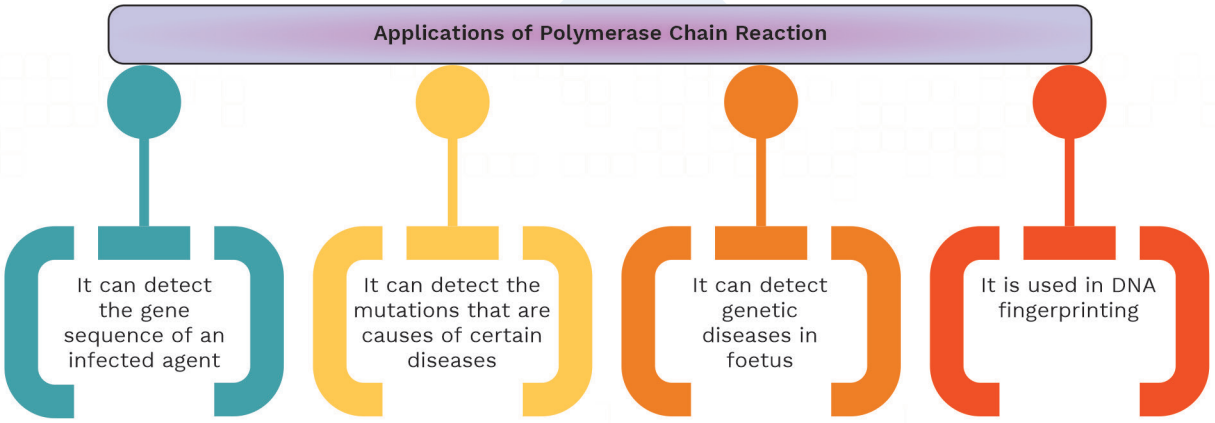
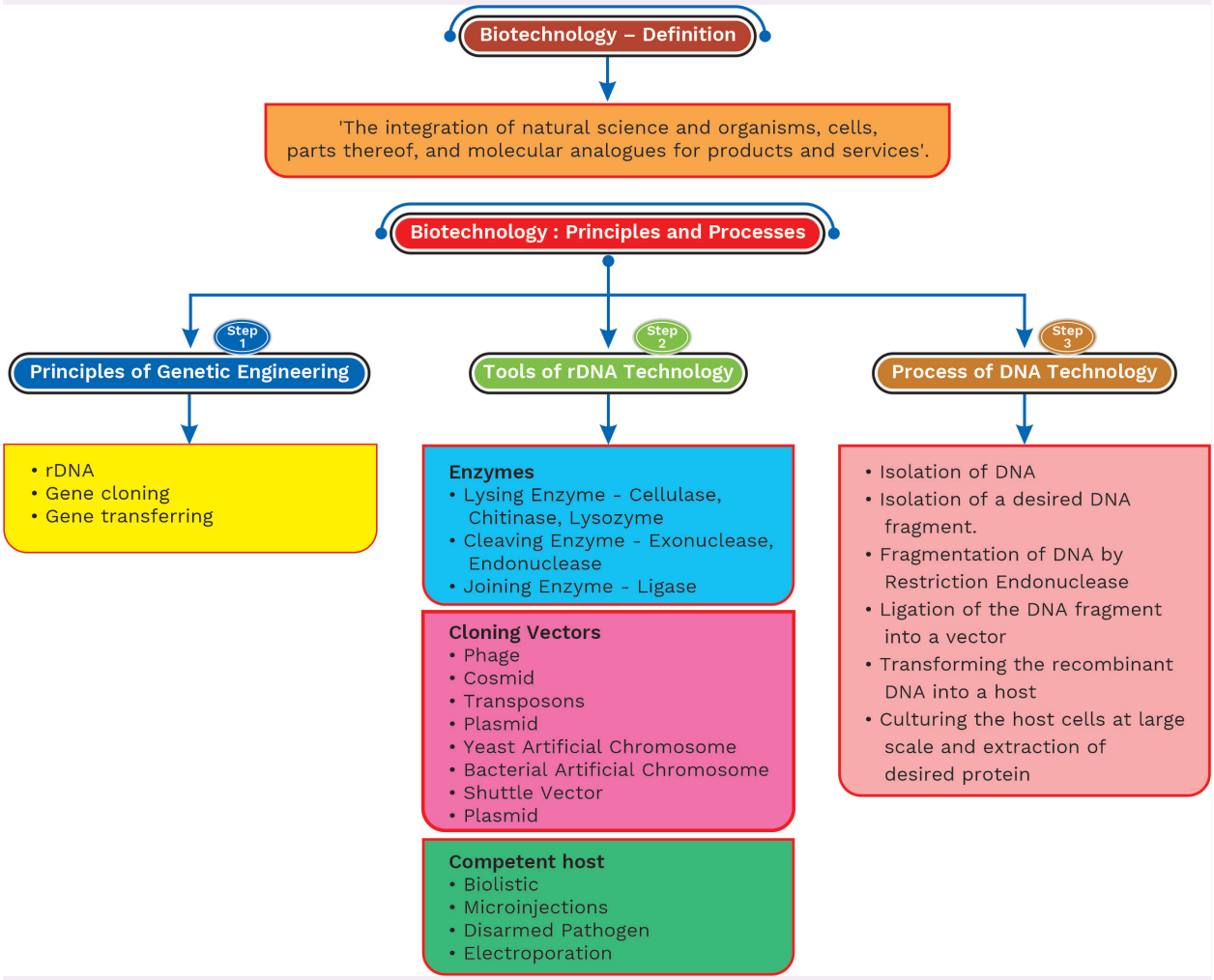


FIG. POLYMERASE CHAIN REACTION (PCR)

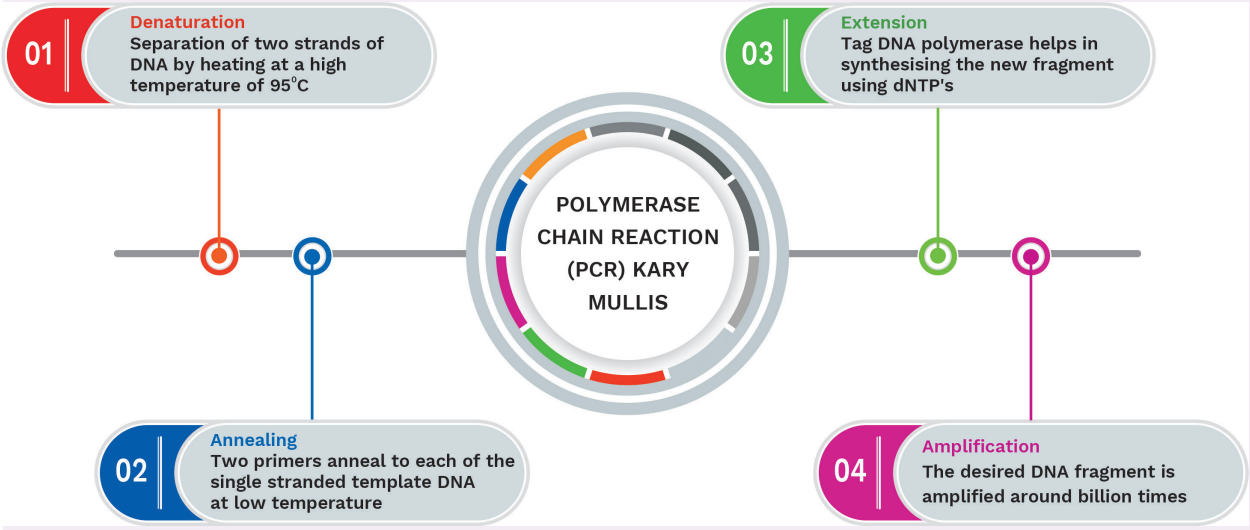
Applications of Polymerase Chain Reaction



Summary


Biotechnology – Definition
'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.
Biotechnology : Principles and Processes
Step 1
Principles of Genetic Engineering
• rDNA
• Gene cloning
• Gene transferring
Step 2
Tools of rDNA Technology
Enzymes
• Lysing Enzyme - Cellulase, Chitinase, Lysozyme
• Cleaving Enzyme - Exonuclease, Endonuclease
• Joining Enzyme - Ligase
Cloning Vectors
• Phage
• Cosmid
• Transposons
• Plasmid
• Yeast Artificial Chromosome
• Bacterial Artificial Chromosome
• Shuttle Vector
• Plasmid
Competent host
• Biolistic
• Microinjections
• Disarmed Pathogen
• Electroporation
Step 3
Process of DNA Technology
• Isolation of DNA
• Isolation of a desired DNA fragment.
• Fragmentation of DNA by Restriction Endonuclease
• Ligation of the DNA fragment into a vector
• Transforming the recombinant DNA into a host
• Culturing the host cells at large scale and extraction of desired protein

Summary


01
Denaturation
Separation of two strands of DNA by heating at a high temperature of 95°C
02
Annealing
Two primers anneal to each of the single stranded template DNA at low temperature
03
Extension
Tag DNA polymerase helps in synthesising the new fragment using dNTP's
04
Amplification
The desired DNA fragment is amplified around billion times
POLYMERASE CHAIN REACTION (PCR) KARY MULLIS

Solved Exercise

Q1 **Two bacteria found to be very useful in genetic engineering experiments are**
(1) *Nitrobacter* and *Azotobacter* **(2) *Rhizobium* and *Diplococcus***
(3) *Nitrosomonas* and *Kliebsiella* **(4) *Escherichia* and *Agrobacterium***

A1 **(4)**
Both act as vectors for cloning the desired DNA and *Agrobacterium* is used for DNA transfer.

Q2 **Restriction endonucleases are**
(1) used for in vitro DNA synthesis
(2) used in genetic engineering
(3) synthesized by bacteria
(4) present in mammalian cells for degradation of DNA

A2 **(2)**
Used for cutting the DNA fragments .

Q3 **Genetic engineering is possible, because**
(1) we can cut DNA at specific sites by endonucleases like DNase I
(2) restriction endonucleases purified from bacteria can be used in vitro
(3) the phenomenon of transduction in bacteria is well underwood
(4) we can see DNA by electron microscope

A3 **(2)**
Restriction nucleases help in cutting DNA at specific sites and removing DNA with desired gene.

Q4 **The restriction enzymes are used in genetic engineering, because**
(1) they can cut DNA at specific base sequence
(2) they are nucleases that cut DNA at variable sites
(3) they can degrade harmful proteins
(4) they can join different DNA fragments

A4 **(1)**
They can cut DNA at specific sites and thus help in further process of genetic engineering.

Q5 **Which of the following organelles is related with genetic engineering?**
(1) Mitochondria
(2) Plasmids
(3) Golgi bodies
(4) Lysosomes

A5 **(2)**
Extra-chromosomal structure that helps in cloning DNA

Q6 **Plasmid has been used as vector because**
(1) it is circular DNA which have capacity to join to eukaryotic DNA
(2) it can move between prokaryotic and eukaryotic cells
(3) both ends show replication
(4) it has antibiotic resistance gene

A6 **(1)**
It can join to any eukaryotic DNA and hence helps in making copies of it.

Q7 **The process of replication in plasmid DNA, other than initiation, is controlled by**
(1) mitochondrial gene
(2) plasmid gene
(3) bacterial gene
(4) none of these

A7 **(3)**
The plasmid gene has the ability to control the initiation while other steps of replication are controlled by the bacterial gene.

Q8 **Which of the following is related to genetic engineering?**
(1) Heterosis
(2) Mutation
(3) Plastid
(4) Plasmid

A8 **(4)**
It acts as a cloning vector.

Q9 **Elution process is involved in**
(1) PCR
(2) Gel Electrophoresis
(3) Separation of DNA from the cell
(4) Replication of the plasmid

A9 **(2)**
Separation of desired DNA fragment from agarose gel is known as Elution.

Q10 **The linking of antibiotic resistance gene with the plasmid vector became possible with**
(1) DNA polymerase
(2) exonucleases
(3) DNA ligase
(4) endonucleases

A10 **(3)**
It helps in joining strands together.