Chapter-11

Biotechnology : principles and processes.

Biotechnology:

Deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.
e.g: making curd, bread, wine

Principles of Biotechnology:

1) Genetic engineering:
Technique to alter the chemistry of genetic material (DNA and RNA) to introduce these into host organisms and thus change the phenotype of the host organism.

2) Bioprocess engineering -
maintainance of sterile ambience in chemical engineering process to enable growth of only the desired microbes

- Technique of genetic engineering include, recombinant DNA, gene cloning, gene transfer.
- In chromosome there is a specific DNA sequence called the origin of replication, which is responsible for initiation of replication.
- Restriction enzymes - molecular scissors - cut piece of DNA. This piece of DNA again linked with plasmid DNA. These plasmid DNA act as a vector to transfer the piece of DNA attached to it.
- DNA ligas - act on cut DNA molecules and join their ends.
- This makes a new combination of circular autonomously replicating DNA created in vitro and is known as recombinant DNA.
- Basic steps in genetically modifying organism.
  i) Identification of DNA with desirable genes.
  ii) Introduction of the identified DNA into the host.
  iii) Maintainance of Introduced DNA into the host & transfer of

DNA to its progeny.

Tools of recombinant DNA technology:

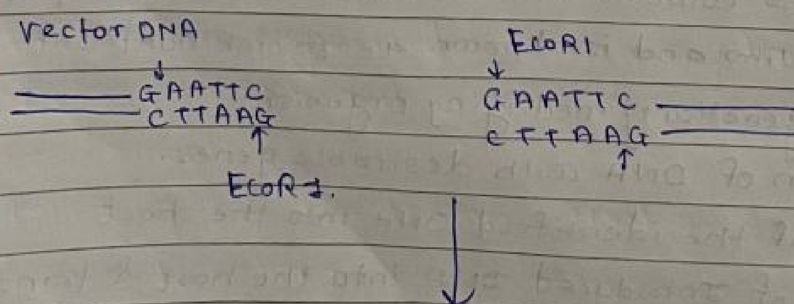
Restriction enzymes:

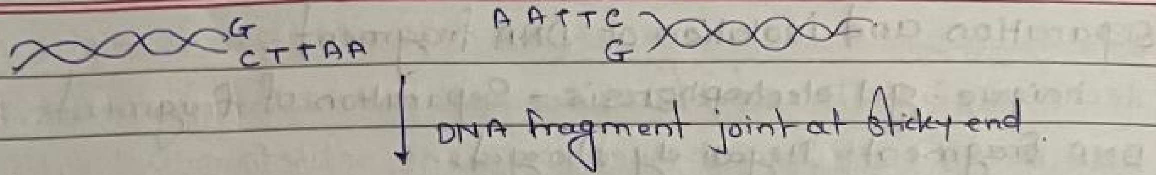
- In year 1963, the two enzymes responsible for restricting the growth of bacteriophage in E.coli were isolated. One of these added methyl group to DNA, while the other cut DNA. The later was called restriction endonuclease.
- First restriction endonuclease – Hind II – recognise six base pair sequence.
- today more than 900 restriction enzymes have been isolated.
- Naming –
  i) First three letters come from genus
  ii) second two letter – species of prokaryote from which they are isolated.

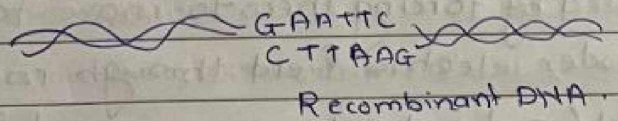
  e.g. ECoRI – comes from Escherichia coli RY 13.
  Here R – derived from strain. roman number – order in which enzyme is isolated.
- Restriction enzyme belong to class nuclease.

Nuclease
- Exonuclease – Remove nucleotide from the end of the DNA.
- Endonuclease – makes cut at specific positions with DNA

- Each restriction endonuclease recognise a specific palindromic nucleotide sequences in the DNA.







Recombinant DNA.

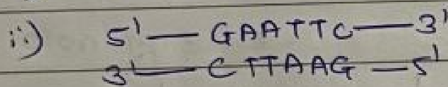
palindrome:

These are group of letters that ~~from~~ form words when read both forward and backword.

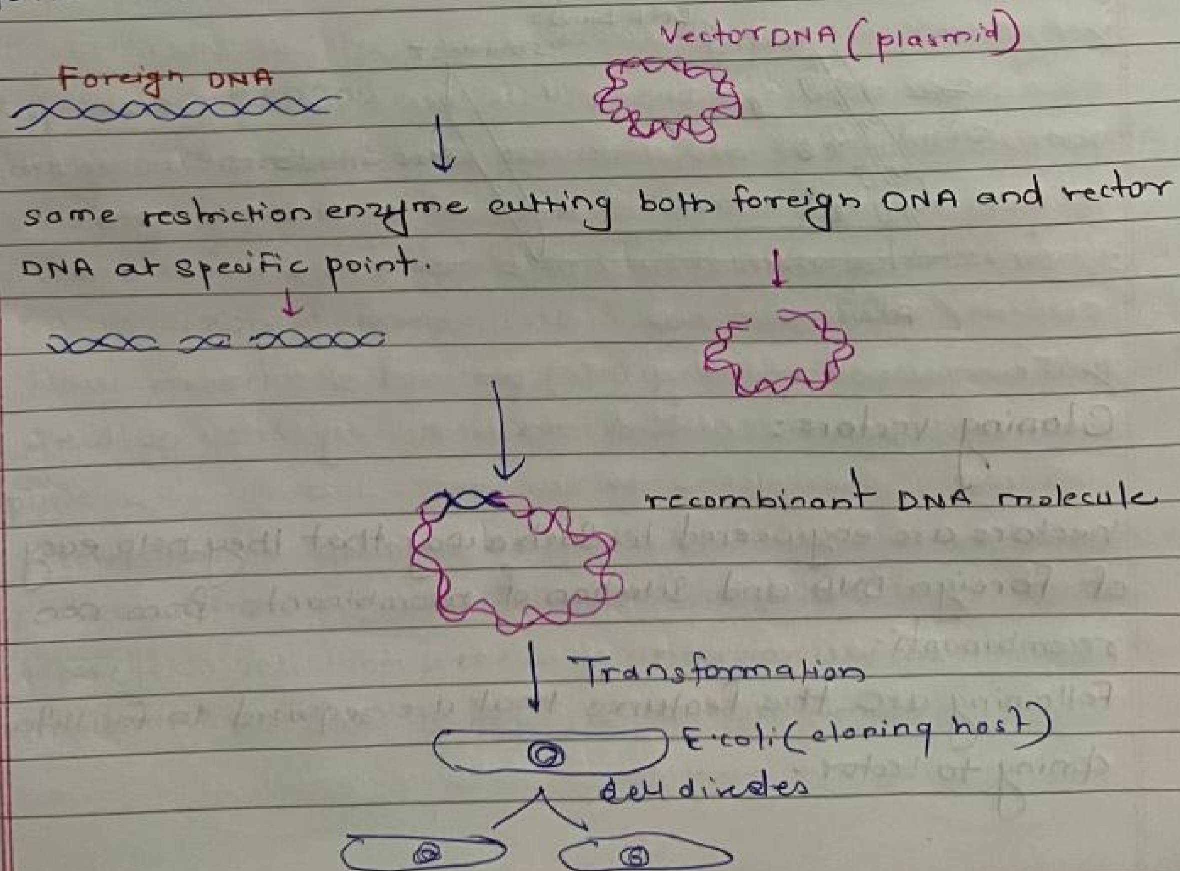
e.g: i) MALAYALAM.

ii) 5' — GAATTC — 3'
3' — CTTAAG — 5'

- Restriction enzyme - cut the strand of DNA a little away from the centre of the palindrome site but between the same bases on the opposite strand.
- There are overhanging stretches called sticky ends on each side.
- Restriction endonuclease - Used in genetic engineering to form recombinant molecule of DNA.



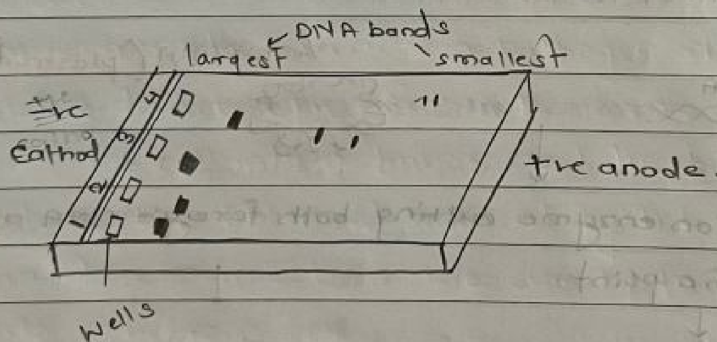
Vector DNA (plasmid)



Separation and isolation of DNA fragment-

- technique - gel electrophoresis - Separation of fragments.
- DNA fragment - Negatively charged.
- Hence it is separated by forcing them to move towards anode (+vely charged) under electric field through medium/matrix.
- matrix - agarose gel (natural polymer extracted from sea weed)
- agarose gel provide sieving effect (pores are formed) through sieve DNA fragment travel and get separated according to size.
- Smaller fragment - move faster - cover more distance.
- large fragment - move slowly - cover less distance.

- Normal DNA fragment - cannot be visualised.
- DNA fragment stained by EtBr (Ethidiumbromide) followed by exposure to UV radiations.
- bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light.
- Elution: Separted DNA fragments are extracted from gel piece.



Cloning vectors:

vectors are engineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non recombinants.

Following are the features that are required to facilitate cloning to vector.

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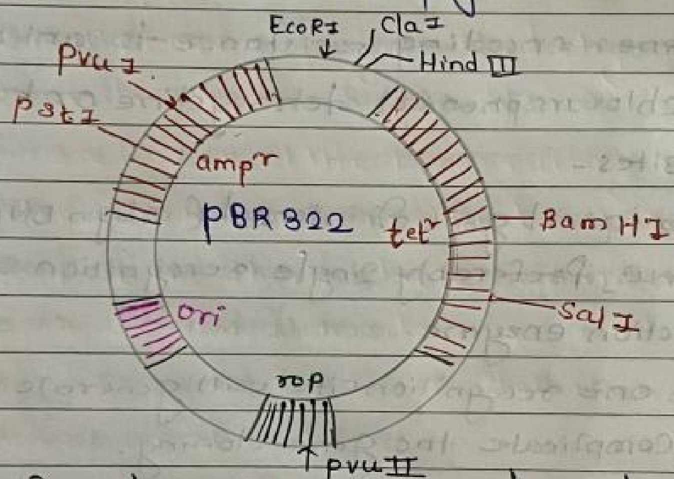
i) origin of replication:- (ori)
sequence from where replication starts. This sequence is responsible for controlling the copy number of the linked DNA.

ii) selectable marker -
help in identifying and eliminating non transformants and selectively permitting the growth of transformants.
Transformation is a process through which piece of DNA is introduced in a host bacterium.
normally genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin.

iii) cloning sites -
- In order to insert gene of interest (align DNA) vector need to have very few, Preferably single recognition site for commonly used restriction enzyme.
- more than one recognition site will generate many fragment which will complicate the gene cloning.
- addition of gene of interest (DNA) is carried out at a restriction site present in one of the two antibiotic resistance genes.

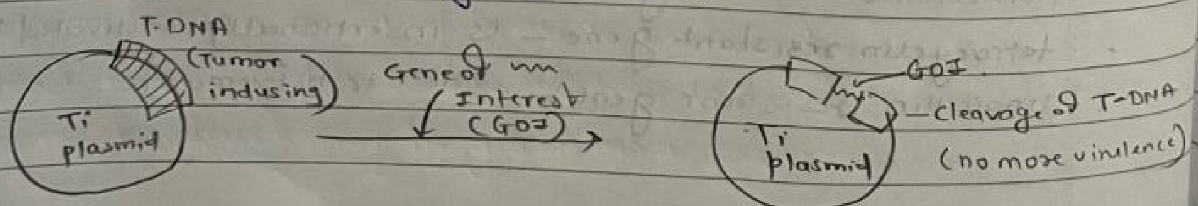
e.g: vector pBR322.
- foreign DNA can be ligated at the Bam HI site of tetracyclin resistance gene in the vector pBR322.
- Now recombinant plasmid will lose tetracyclin resistance due to insertion of foreign DNA
- Now vector has become tetracyclin sensitive. so it will not grow on tetracyclin medium. but it will grow on ampicillin plate.
- recombinant - grow on ampicillin plate but not on tetracyclin.
  non recombinant - grow on both the plates.
- tetracyclin resistant gene — is insertionally inactivated by BamH I
- ampicillin resistant gene — select transformants.

- alternative selectable markers differentiates recombinants from non recombinants on the basis of their ability to produce colour in the presence of chromogenic substrate
Here recombinant DNA is inserted within the coding sequence of an enzyme, β-galactosidase. results in inactivation of gene for synthesis of enzyme is called insertional inactivation.
- Normal chromogenic substrate - gives blue coloured colonies.
- After insertional inactivation of β-galactosidase gene - no coloured colonies.



iv) vectors for cloning genes in plants and animals.

1) Agrobacterium tumifaciens a pathogen of several dicot plants is able to deliver a piece of DNA known as 'T-DNA' to transform normal plant cell into a tumor. and direct these tumor cell to produce the chemical required by the pathogen.
2) animal retrovirus have the ability to transform normal cell into cancerous cell.
3) The tumor inducing (Ti) plasmid of agrobacterium tumifaciens has now been modified into a cloning vector which is no more pathogenic to the plants but deliver gene of interest to plant. Similary retrovirus is also disarmed and used to deliver gene of interest.



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Competent host (for transformation with Recombinant DNA)

1. • DNA is treated with specific divalent cation such as calcium which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.
   • Now incubate recombinant DNA cells on ice (cold shock) and then at 42°c (Heat shock) & then again on ice this enables the bacteria to take up recombinant DNA.
2. Microinjection - recombinant DNA is directly injected into the nucleus of an animal cell.
3. gene gun / Biolistics - cell are bombard with high velocity micro-particles of gold or tungsten coated with DNA
4. Use of Disarmed pathogen - infect host cell & transfer recombinant DNA.

processing of recombinant DNA technology :

i) Isolation of genetic material -

- To break cells and release the DNA along with other macromolecules such as RNA, protein, polysaccharide and also lipids. achieved by ^treating cells or tissue with enzymes such as lysozyme (for bacteria), cellulase (for plant cell), chitinase (for fungus)
- Now RNA removed by treatment with ribonuclease, protein removed by treatment with protease.
- Now purified DNA precipitates out after addition of chilled ethanol. This can be seen as collection of fine threads in the suspension.

ii) Cutting of DNA at specific location -

- Incubate purified DNA molecule with the restriction enzyme
- Incu at optimal condition for the specific enzyme.
- perform agarose gel electrophoresis to check the progression of a restriction enzyme digestion. DNA (-vely charge) ⟶ move to anode (+vely charge)

• for joining ligase enzyme is used. DNA ligase join gene of interest with vector. This results in the preparation of recombinant DNA.

iii) Amplification of gene of interest using PCR:

- PCR - polymerase chain reaction.
- multiple copies of gene of interest are synthesised in vitro
- Requirement - 2 sets of primer, DNA polymerase (isolated from Thermus aquaticus)
- primer - small chemically synthesised oligonucleotide that are complementary to the region of DNA.
- DNA polymerase - extend the primer using nucleotides
- Many cycles of PCR - DNA amplified to billion times.
- DNA polymerase is thermostable remain active during high temperature induced denaturation of double stranded DNA.

iv) Insertion of recombinant DNA into the host cell -

If a recombinant DNA bearing gene for resistance to an antibiotic (e.g. ~~pen~~ ampicillin) is transferred into E.coli cell, the host cell become transformed into ampicillin resistant cell. If we spread the transformed cell on agar plate containing ampicillin ~~resistant~~ cell only transformed will grow, untransformed recipient cell will die. Since due to ampicillin resistant gene, one is able to select a transformed cell in the presence of ampicillin. ampicillin resistance gene in case is selectable marker.

v) obtaining the foreign gene product -

- If any protein encoding gene is expressed in a heterologous host it is called a recombinant protein.
- Gene of interest may be grown on a small scale in the laboratory

- To produce large quantities development of bioreactors where large volumes (10-1000 litre) of culture canbe processed
- bioreactor is a vessel in which raw material are biologically into specific products. biorector provide optimal condition for achieving the desired product by providing optimum growth conditions. (temperature, pH, substrate, salts, vitamins, oxygen).
- most commonly used bioreactor - stirred type.

Usually cylindrical with a curved base to facillitate the mixing of the reactor content; make oxygen availability throughout the bioreactor.

bioreactor has - agitation system, oxygen delivery system, foam control system, temperature control system. pH control system, sampling port so that small volumes of the culture can be withdrawn periodically.

Downstream processing:

It include separation and purification.

The product has to be formulated with suitable preservative

Such formulation has to undergo through clinical trials as in case of drugs.

Strict quality control testing for each product is also required.

The downstream processing and quality control testing vary from product to product.