Biotechnology

Biotechnology may defined as the use of microorganism or plant or animals or their part to generate product and services useful to human beings/the process involves genetic engineering

- The products-bt crops,vaccines,rDNA vaccines etc

Principles of Biotechnology

- Two principles
  [1] Genetic Engineering
  [2] the tech that grow and facillate growth of microbs/Host
- Genetic Eng [def-tech to alter sequence of genetic material,so change **phenotype**] includes

1. Creation of r DNA
2. Gene cloning
3. Gene transfer

Tools of Biotechnology/Recombinent DNA technology

- The tools of are
  1. Restriction Enzyme
  2. Ligase
  3. Vector
  4. Host
- **Restriction enzymes are**
  1. They serve as the tools or cutting DNA molecule at palindromic site
  2. This is class of nuclease enzyme
  3. May be endo or exo
  4. Linn & Arber 1963 two enzyme from coli that can restrict the growth of phage
  5. First was Hind II
  6. Functioning depends up on spl site called Recognisation site that is **palindromic nucleotide sequence in the DNA GAATTC**
  7. They cuts at PNS, called cleavage, and leaves G and C called **sticky ends**

8. The above forms H bond with their complementary counterpart
9. The cut peaces are separated by **Gel electrophoresis**
10. Nomenclature [First word from the genus+ Two word from species +the site where they attack e.g. **Eco HYRI**]

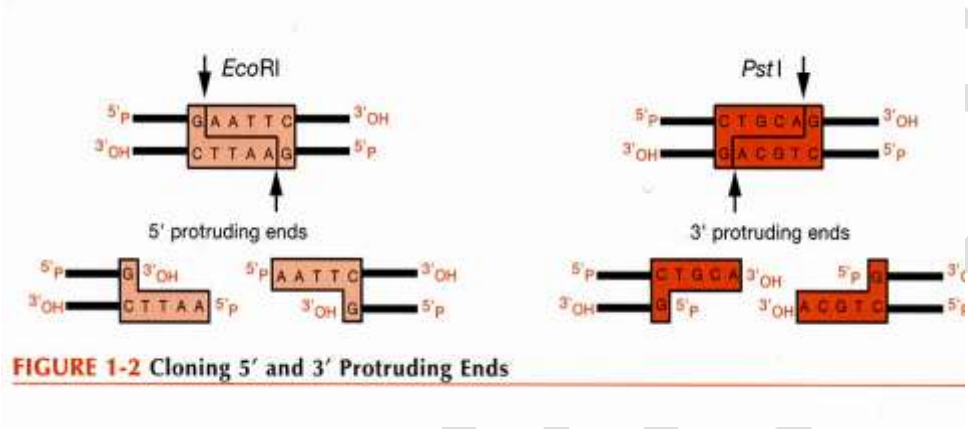
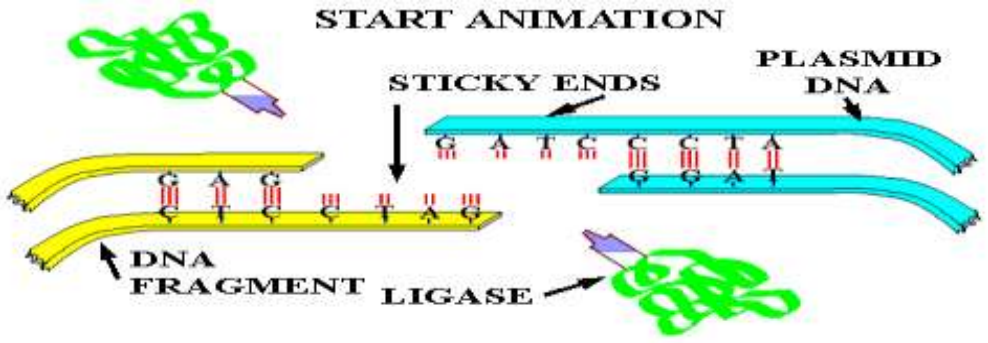
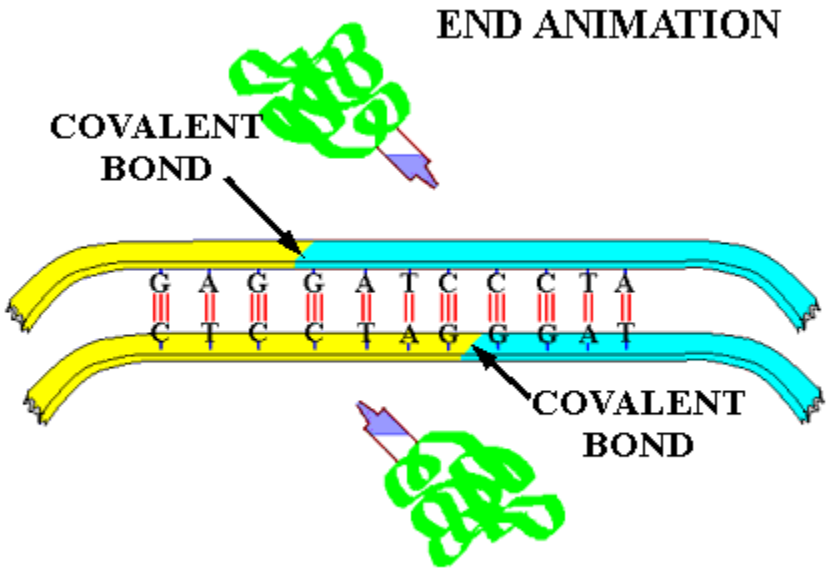


FIGURE 1-2 Cloning 5′ and 3′ Protruding Ends

- **LIGASE**

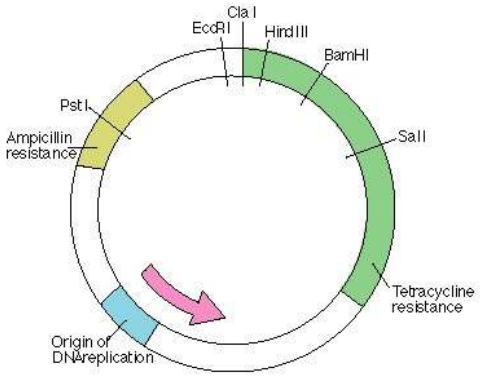
1. an enzyme commonly used in molecular biology laboratories to join together DNA fragments.
2. **Also called synthetases**, because they are used to synthesize new molecules.
3. **Example DNA ligase**





- **VECTORES**

1. A vector is a DNA molecule which have the self replication ability in host cell [e.g.phage and plasmid in bacterial cell]or into which DNA fragment to be cloned **DNA insert**
2. **A** good vector must have
   a. Autonomous replication
   b. Small size
   c. Easy to isolate and purify
   d. Easy to introduced in host cell
   e. Easy linking to foreign DNA



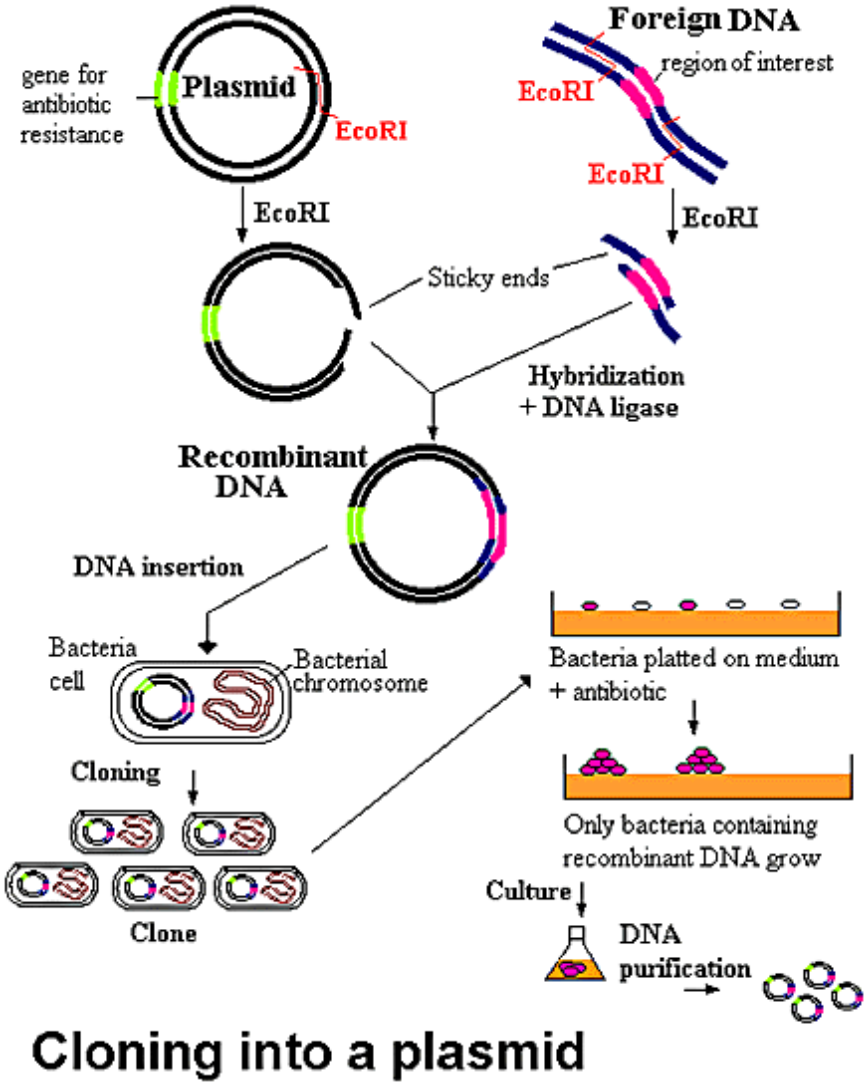
3.Features that are required to facillate cloning-

a. **Origin of Replication,ori,***the sequence from where the replication starts and any pcs of DNA when linked to this seq. can self replicate in host cell. [ii]responsible for the* ***copy number***
b. **Selectable Marker is a gene** which *helps in identification of those host cell which have vector[**Transformant]** and elimination of non transformants* .Thus provide selected **transformation[the process by which a DNA pc introduced to hot bacterium]mostly antibiotic resistence gene, as tetracycline or galactoside,are good marker**
c. C**loning sites/Recognisation site./*.identification of site***
    - *the site to which an alien DNA will linked*
    - *may and may be more than one.*
    - *The ligation is carried out at a* ***restriction site*** *present in one of the two antibiotic resistance genes*
    - E.g. we can ligate alien DNA to at restriction site BamH I site of tetracycline resistance gene in vector pBR322.
    - The r plasmid will lose antibiotic resistance due to alien DNA
    - Then it placed in ampicillin plate
    - Then again it placed in tetracycline medium
    - Those which grows in ampicillin and tetracycline medium are non transformants whilst which grows only in ampicilline are transformants
    - **Thus antibiotic gene helps in selecting transformants so called marker**
    - **Another method is based on color-**r DNA is inserted into the coddding sequence of beta galactoside which result inactivation of enzyme[**insertional inactivation]** produce blue color are non transformonts whilst which gives no color

Process of R DNA tech

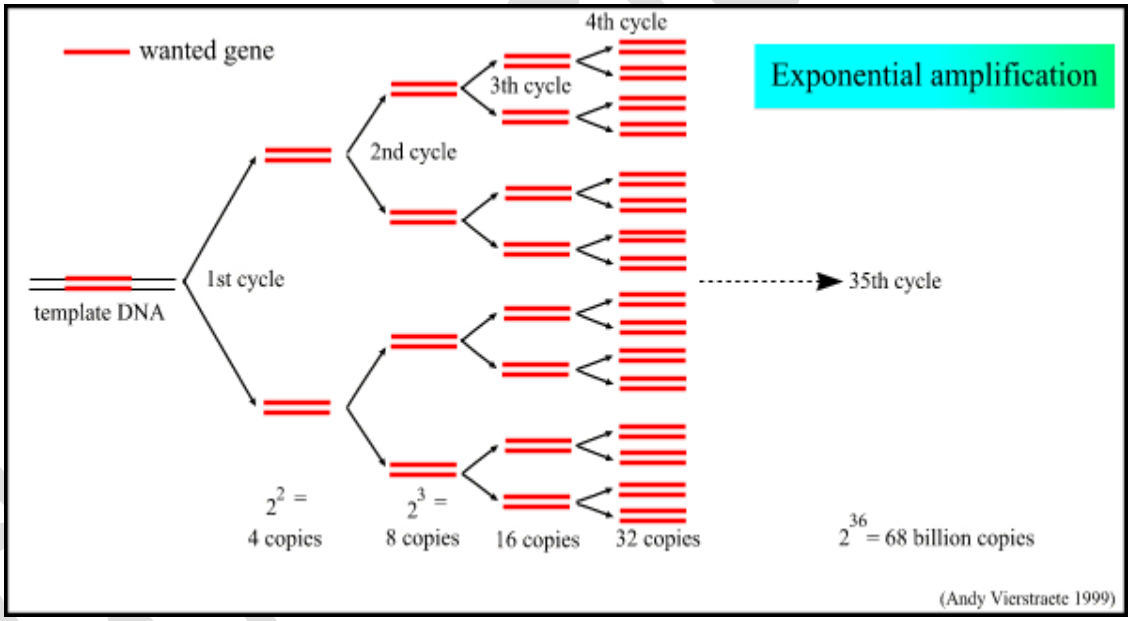
➢ *Isolation of Genetic Material*

1. Break the cell to release DNA[with RNA proteinsetc]
2. Cell is broken down by treating the cell by Lysozyme,Cellulose,Chitinase
3. Purified
   - RNA removed by treating ribonuclease
   - Proteins removed by treating Protease
   - And so on,Finally purified by C_2H_5OH



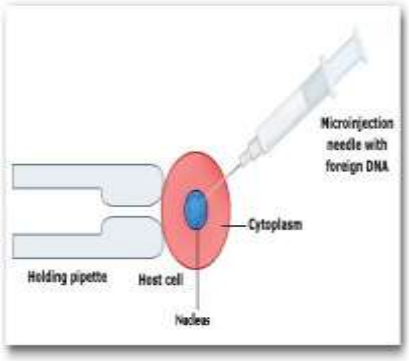
Cloning into a plasmid

- ***Clevage at specific site[by Nuclease]***
- Isolation of the desired DNA [GENE]fragment by ***agrose gel electrophoresis***
- ***Amplification of gene by PCR***
  1. Amplification mean making of multiple copies of desired gene
  2. PCR invented by K mullis noble for Medicine
  3. **PCR involves**
     - Denaturation[by heating]
     - Primer annealing
       - Primers are chemically synthesized peaces of DNA which are complimentary to the desired DNA
       - Two sets of primers are used
       - DNA Pol II is the enzyme used to making copies of DNA by the use of DNA and its primers
     - Extension of primers

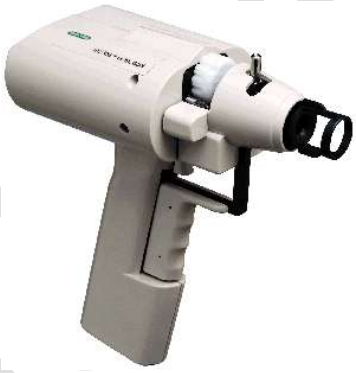


- ***Ligation of DNA fragment to vector by ligase which result r DNA***
- ***Transfering of r DNA to Host***
  - The r DNA can't insert to host cell because it is hydrophobic in nature and hydrophobic molecule pass through cell membrane by ion selective method

- Hence to make Bacterialcompetent to take up DNA,Bacteria is treated with Calcium
- **Now** r DNA can inserted to bacteria at 42^0C
- However, now a days,r DNA can injected directly by gene gun or microinjection
  - Gene gun is a suitable method for plant cell where cells are bombard with high velocity coated DNA{coated by Tungsten or gold}



Microinjection



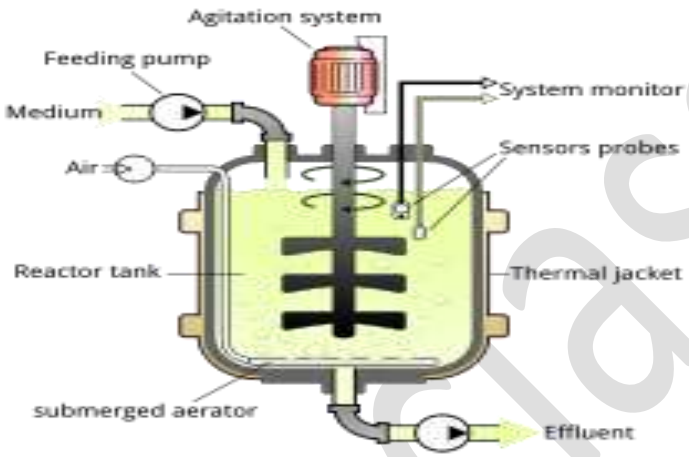
gene gun

- ***Culture of host in medium***
  - The host is cultured in suitable medium
  - The gene cloning process occur
- Obtaining the foreign product [desired] e.g. vaccine by bioreactors
- Downstream [separation and purification, clinical trials] process of the product as a finished product ready for marketing

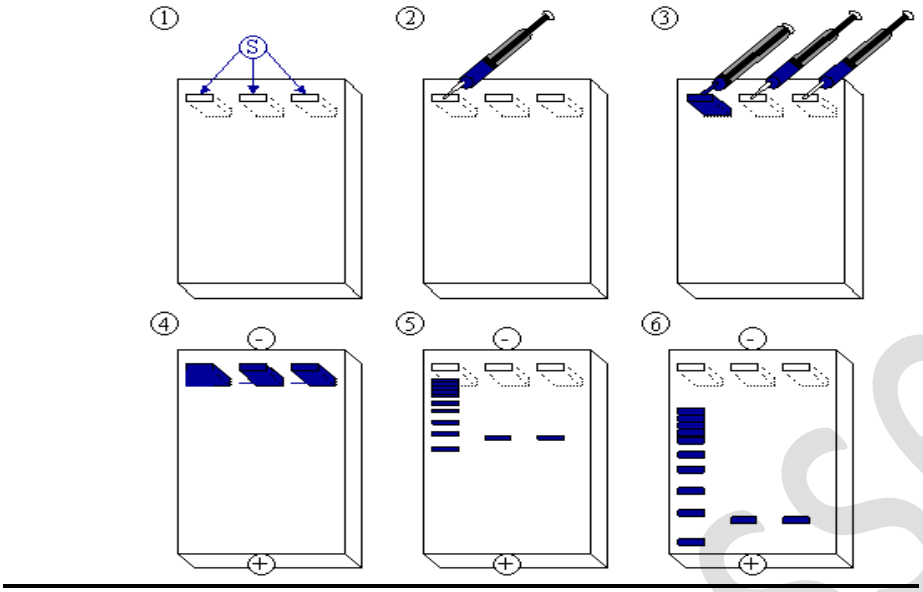
Bioreactors

- This is a special type of vessel in which raw material is converted into desired product by microbes.
- This provide optimum growth conditions
- Out of them stirred bioreactor are most common-
  - The stirrer facillate the mixing and oxygen availability through the reactor

- This have following part
  - Agitation system
  - Oxygen[air] delivery system
  - Reactor tank
  - Thermal jacket etc.



- ***Gel electrophoresis***
  - Separation of charged molecules in an electric field.
  - Nucleic acids have 1 charged phosphate (- charge) per nucleotide.
  - Separation based on length: longer molecules move slower.
  - Done in a gel matrix to stabilize: agarose or acrylamide.
  - Stain with EtBr intercalates between DNA bases and fluoresces orange.
  - Run alongside standards of known sizes to get lengths



Some other important point

PLASMID

- Extrachromosomal, double-stranded, usually circular, supercoiled DNA molecules
- Found in many bacterial species
- Replicate and are inherited independently of the bacterial chromosome
- Maintain copy number in cell through an origin of replication (replicon)
- Usually have genes coding for enzymes that provide benefits for the host bacterium, eg. antibiotic resistance

COPY NUMBER

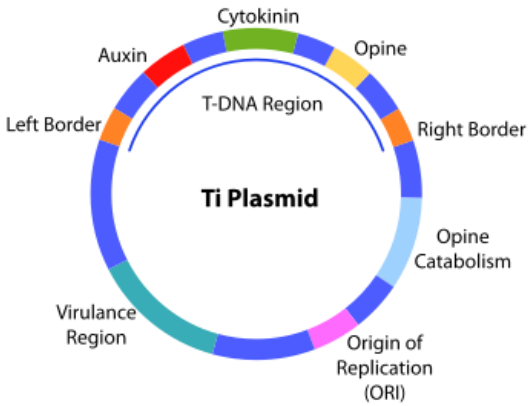
- The copy number reflects the average number of copies of a certain plasmid inside a host cell. The higher the copy number, the more efficient the plasmid is at replicating itself.
- Researchers using plasmids as vectors usually choose high copy number plasmids as their vectors since you can get a large number of plasmids from relatively fewer cells in less time.

cDNA

- In genetics, **complementary DNA** (**cDNA**) is DNA synthesized from a messenger RNA (mRNA) template in a reaction catalysed by the enzymes reverse transcriptase andDNA polymerase.
- cDNA is often used to clone eukaryotic genes in prokaryotes.
- When scientists want to express a specific protein in a cell that does not normallyexpress that protein they will transfer the cDNA that codes for the protein to the recipient cell.
- cDNA is also produced by retroviruses (such as HIV-1, HIV-2, Simian Immunodeficiency Virus, etc.) which is integrated into its host's genome where it creates a provirus

Ti plasmid

- A **Ti** or **tumour inducing** plasmid is a circular plasmid that often, but not always, is a part of the genetic equipment that *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* use to transduce its genetic material to plants.
- The Ti plasmid is lost when *Agrobacterium*is grown above 28°C. Such cured bacteria do not induce crown galls, i.e. they become avirulent
- The plasmid has 196 genes that code for 195 proteins. There is no one structural RNA. The plasmid is 206,479 nucleotides long, the GC content is 56% and 81% of the material is coding genes. There are nopseudogenes.



ASSIGNMENT-1

1. Each nuclease enzyme cut DNA at its specific site. Name them?
2. What are the technique included under biotechnology?
3. Write a note on gel electrophoresis?
4. Define plasmid?
5. Explain with refrence to PCR
    a. A specific enzyme helps in amplification in PCR.Name the bacterium from which it isisolated?
    b. Explain its use in moleculer diagnosis?

ASSIGNMENT-2

1. What is the significance of palindromic sequence?
2. Name the enzyme that cuts DNA sequence at A and G
3. Write digramatically the three sequence of PCR?
4. Why a cell must be competent to introduce rDNA?
5. Represent digramatically the steps in the formation of r DNA by EcoRI?

ASSIGNMENT-3

1. A plasmid and a DNA sequence in a cell need to be cut for producing rDNA.What name is given to the enzyme that will work as moleculer scissors?
2. Mention the function of DNA polymerase and DNA ligase?
3. Which of the following will produce sticky ends-

4. DNA cannot pass through cell membrane. why?
5. Mention three ways to introduce rDNA into Host cell?

ASSIGNMENT-4

1. Define biolistic?
2. Describe the isolation of DNA for r DNA?
3. Define elution?
4. Draw stirred bioreactor?
5. Define plasmid?Mention the role of vectores in r DNA tech?

ASSIGNMENT-5

1. Show the stepd of rDNA only by diagram?
2. Name the technique used to seprateDNA fragments in lab?

3. Give the chemical structure of nucleotide?
4. Explain the importence of *ori,am^R,and rop in pBR322?*
5. Explain selectable markers?
6. Explain cloning sites?
7. What is the role of lysozymes,chitinase etc?
8. One or few sites are preferred in a vector as recognisation site.Why?
9. Define
    a. Electroporation
    b. Palindromes
    c. Transgenic
    d. Bioconversion
    e. R protein
    f. Microinjection
    g. Translation

REFRENCES

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