L.11 Genetic engineering

Introduction:

Genetic engineering: Is the use of genetic knowledge to artificially manipulate genes by many technological processes. One of these processes is the cloning of a gene or called recombinant DNA technology .It can produce cells that contain a foreign gene that are capable of producing a new and different protein.

There are three goals for r DNA technology:

1. Eliminate undesirable phenotypic traits
2. Combine beneficial traits of two or more organisms
3. Create organisms that synthesize products humans need

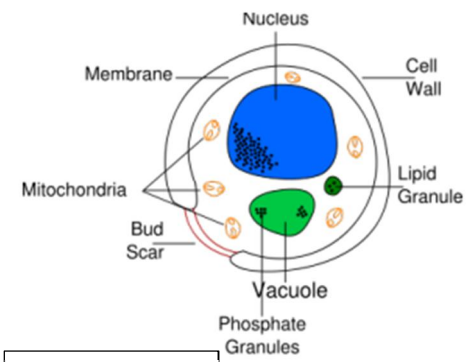
Recombinant DNA technology:

Recombinant DNA technology adds genes from one type of organism to the genome of another**.**

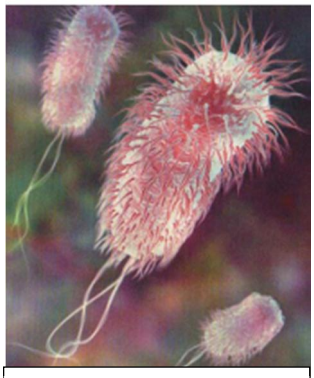
Genome: The overall genetic structure of an organism.

Recombinant DNA technology requires restriction enzymes that cut donor and recipient DNA at the same sequence; DNA to carry the donor DNA called cloning vectors; and recipient cells (host cells)

Host cells: either prokaryotic (bacteria) or eukaryotic organism (yeast or cell line) . The organisms most frequently used in recombinant DNA research is the bacterium ***Escherichia coli*** for many reasons: the bacteria are haploid, reproduced asexually by binary fission and have two mutant strains.



Yeast cell

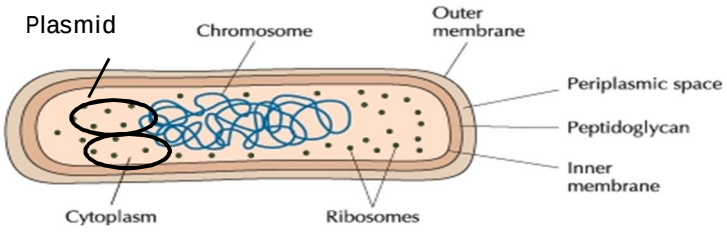


Bacterial cell

A technician often begins by selecting a **vector** it mean by which rDNA is introduced into a host cell. There are many types of vectors:

1. **Bacterial plasmid vectors**

The plasmid is small circular molecule of DNA found in both prokaryotic and yeast .Its contains one or two genes. A plasmid can be replicated independently of the bacterial chromosome, and it can become integrated in the chromosome. The bacterial chromosome contains all the genes necessary for normal growth and developmental .The genes of the plasmids are not essential, yet they are still replicated in a given cell. They are transmitted through successive generations**.**



They are 2 types of plasmid:

a. **F-plasmid:**

Genes carried on an plasmid code for proteins that promote bacterial conjugation, a process by which one bacterial cell transfer DNA to another . Only a cell having F-plasmid can be a donor called F^+ and a cell lacking an plasmid called F^- ,for example: *Escherichia coli.*

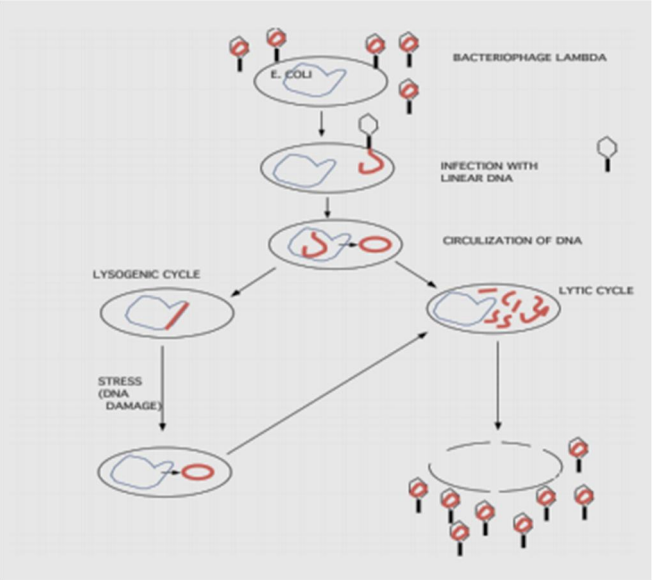
b. **R –plasmid:**

Is composed of one or more genes as before, and can insert itself at random locations in the bacterial chromosome, these genes not essential to bacterial growth but especially important for resistance to the various antibiotics including streptomycin, tetracycline and ampicillin, for example *Shigella* (caused dysentery).

2. **Bacteriophage vectors :**

The other vector used phage such as **lambda**, lambda is most common phage, fragments up to 23 kb can be may be accommodated by a phage vector ,also can integrate their DNA into host chromosomes.

Lambda bacteriophage can spread through a population of *E. coli* and destroy it through repeated cycles of infection, this being called the lytic pathway.



Lytic pathway

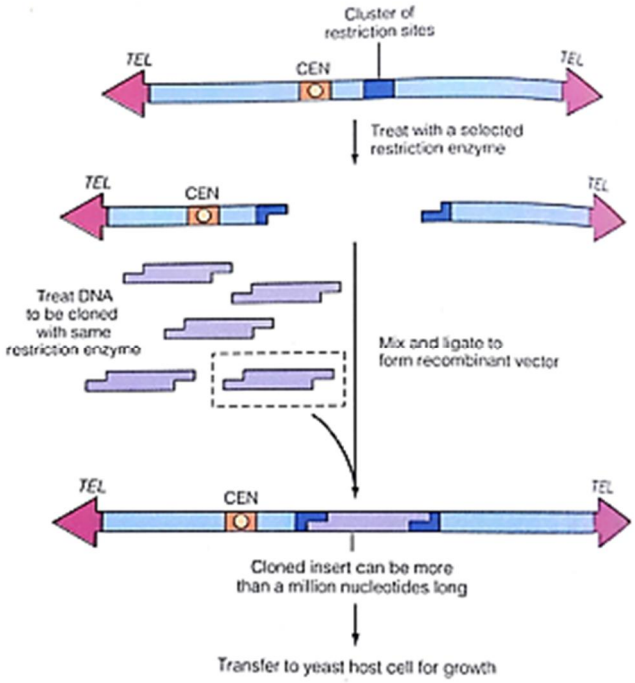
A viral enzyme cuts the bacterial chromosome at specific site, and then the viral DNA is inserted between the cuts and sealed in place. The modified replicated and passed on to succeeding cell generations. Later, the viral DNA may move out of chromosome and an infections cycle begin again.

3. Cosmid vectors:

Fragments from 30 to 46 kb can be accommodated by a cosmid vector. Cosmids combine essential elements of a plasmid and Lambda systems.

4. Yeast Artificial Chromosomes (YAC):

Yac is a vector used to clone large DNA (larger than 100 kb and up to3000).It's an artificially constructed chromosome and contains the telomeric, centromeric ,and replication origin sequences needed for replication and preservation in yeast cells.



Restriction endonuclease enzyme:

The bacteria are using these enzymes to destroy foreign DNA (the DNA of invading viruses) .The enzymes recognize certain nucleotide sequences found on the foreign DNA. There are three types of restriction endonucleases are known:

1.**Type I and III restriction endonucleases**

These are not useful for gene cloning because they cleaved DNA not at recognition site therefore, leading to the random cleavage patterns

2.Type II restriction endonucleases

Recognize specific sites and cleave at just these sites .The sites are recognized by type II endonucleases are inverted repeats: they have two symmetrical folds. Restriction endonucleases are named after the bacteria from which they were isolated .

In a typical recombinant DNA (cloning gene) experiments:

1- The bacterial cells are broken open and the plasmids are separated from larger segments.

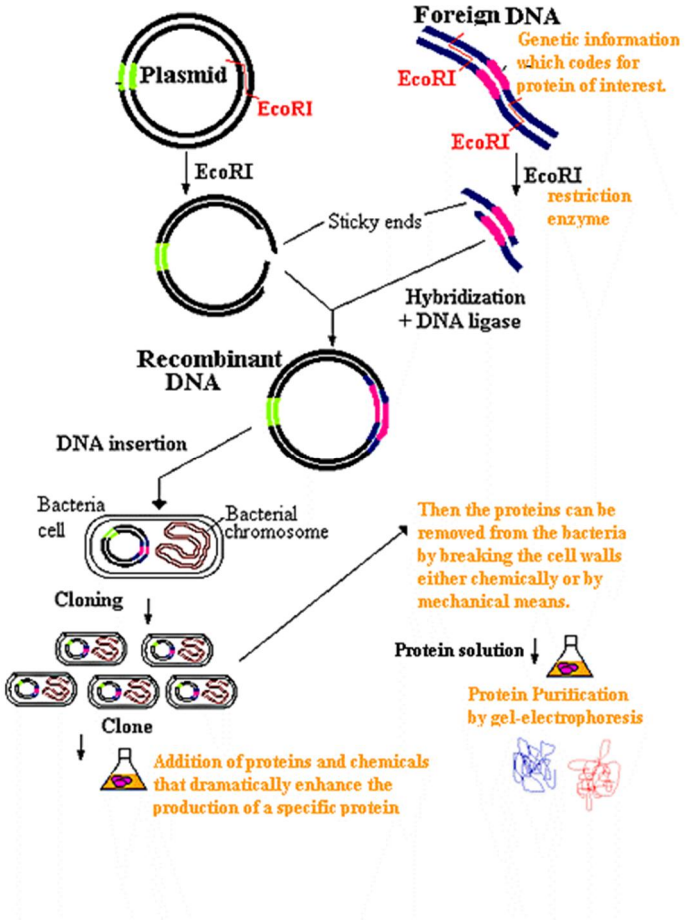
2- Next, the restriction endonucleases enzyme is added to the plasmid suspension, which splits each circular plasmid into one or more linear pieces of DNA.

3- Then it's mixed with fragments of foreign DNA of another organism or in some cases synthesis in laboratory, prepared with the same restriction endonuclease enzyme.

4- Another type of enzyme called **ligase** is add to seal the mixture of a pieces back into circular plasmids, some of which now include the foreign DNA (rDNA).

5- Finally, the reconstituted plasmids are added to a colony of living bacterial cells (usually treated with a calcium salt to make them more permeable).

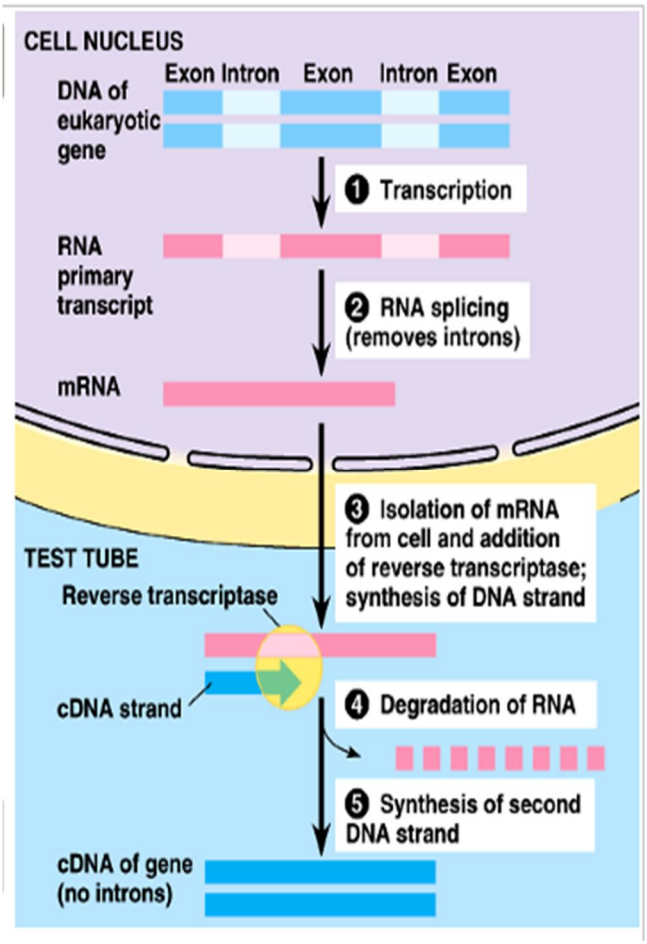
Some of the plasmids are taken up into some of bacterial cells when these transformed cells replicate their DNA prior to cell division. They also replicate rDNA (plasmid containing foreign piece of DNA).This process called cloning of gene (production of copies of genes. So, succeeding generations of this new bacterial strain will have the foreign DNA and with it), or transduction some of vectors into bacterial cell by a phage .



In order form a mammalian gene expression to occur in bacterium, the gene must have to be accompanied by the regulatory regions. Also the gene should not contain introns because bacterial cells do not have the necessary enzymes to process primary mRNA. There are other techniques called cloning gene by reverse transcriptase.

Cloning genes of eukaryotes:

Once the appropriate mRNA has been isolated ,the next step is to produce from it the corresponding single stranded DNA ,using **reverse transcriptase** from a retrovirus, DNA polymerase are then used to replicate the DNA strand to form the double helical structure of the transcript. Then inserting foreign DNA into plasmids are then utilized ,a restriction endonucleases enzyme cut open the plasmid, and a ligase for restores the bonds in the DNA backbone, and the plasmid is inserted into bacterial host by transformation for production of copies of genes.

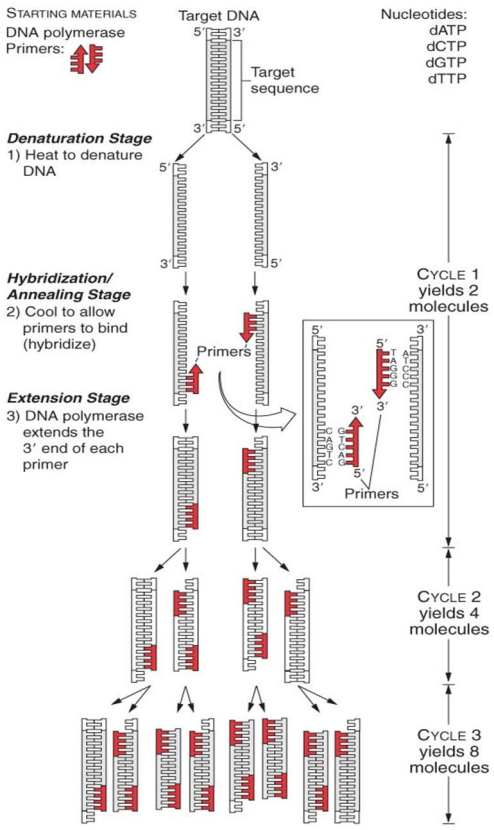


Another common use for reverse transcriptase is to generate DNA copies of RNAs prior to amplifying that DNA by polymerase chain reaction (PCR). Reverse transcription PCR, usually called simply RTPCR, is a stupefying useful tool for such things as cloning cDNAs, diagnosing microbial diseases rapidly and a many of other applications. In most cases, standard preparations of reverse transcriptase are used for RTPCR

Polymerase chain reaction (PCR).

PCR was developed in the 1980s by Kary Mullis .This technique used for making copies, or amplifying, a specific sequence of DNA in a short period of time Process, can be summarized as follows:

1. **Target DNA** to be amplified is added to a tube, mixed with four types of nucleotides (dATP, dCTP, dGTP, dTTP), buffer
2. **Primers** are added – short single-stranded DNA oligonucleotides (20–30bp long) .These are complementary in sequence to opposite ends of the target sequence.
3. **Taq 1,a DNA polymerase** produced by *Thermus aquaticus* ,a microbe that inhibits hot springs. This enzyme makes PCR easy because it does not fall apart when DNA is heated,as most protein do.
4. Reaction tube is placed in an instrument called a thermocycler process.
5. Thermocycler will take DNA through a series of reactions called a **PCR cycle**
6. Each cycle consists of three stages
    1. Denaturation : Heat is used to separate the double helix of target DNA
    2. Annealing : The two short DNA primers bind by complementary base pairing to the separated of each target strand)
    3. Extension : Taq DNA polymerase adds bases to the primers and builds a sequence complementary to the target sequence
    4. At the end of one cycle, the amount of DNA has doubled cycles are repeated 20–30 times .



Advantage of PCR

Ability to amplify millions of copies of target DNA from a very small amount of starting material in a short period of time

Applications

1. Making DNA probes
2. Studying gene expression
3. Detection of viral and bacterial infections
4. Diagnosis of genetic conditions
5. Detection of trace amounts of DNA from tissue found at crime scene

Medical application of genetic engineering:

1. Recombinant DNA methodology has made available large quantities of substances include insulin, interferon (an antiviral agent),growth hormone, growth factors, tissue plasminogen activator (TPA) for dissolving blood clotting , erythropoietin

(EPO) for treating anemia, granulocyte-macrophage colony-stimulating factor (GM-CSF) for stimulating the bone marrow after a bone marrow transplant, angiostatin and endostatin for trials as anti-cancer drugs ,parathyroid hormone and vaccines for diseases such as :hepatitis B and herpes

2. Genetic engineering is making it possible to manufacture antibodies to diagnose and treat diseases.
3. Genetic screening: DNA microarrays used to screen individuals for inherited disease caused by mutation can also identify pathogen's DNA in blood or tissues
4. Gene therapy helps treat genetic disorders and other kinds of disease. At 1990, the in scientist approved gene therapy treatments on people. In the first, a tumor necrosis factor gene was injected into people with cancer. The necrosis factor often shrinks tumors .Also use the gene therapy for aids, hemophilia, cystic fibrosis and diabetes. In general human gene therapy, is a process by which inserting one or more normal genes instead of defective gene into the somatic cells of an organism to correct a genetic defect.
5. DNA finger printing can help solve crime. Identifying individuals or organisms by their unique DNA sequence.
6. Mapping of human genes on chromosomes:

Scientists are able to link mutations and disease states to specific sites on chromosomes.