Recombinant DNA technology

Learning Objectives

- Students should able to understand
  - Discovery
  - Goals and objectives
  - rDNA technology procedure
  - Enzymes
  - Vectors
  - Techniques
  - Applications

Recombinant DNA technology

DNA molecules that are extracted from different sources and chemically joined together; for example DNA comprising ananimal gene may be recombined with DNA from a bacterium

Discovery of recombinant DNA technology

Discovery of DNA structure Watson & Crick in 1953

Isolation of DNA ligase in 1967

Isolation of REase in 1970

Paul Berg generated rDNA technology in 1972

Cohen & Boyer in 1973 produced first plasmid vector capable of being replicated within a bacterial host

Outline of recombinant DNA technology

1.Selection of desire piece of DNA

2. Insertion of selected DNA in to cloning vector

3. Introduction of recombinant vectors into host cells

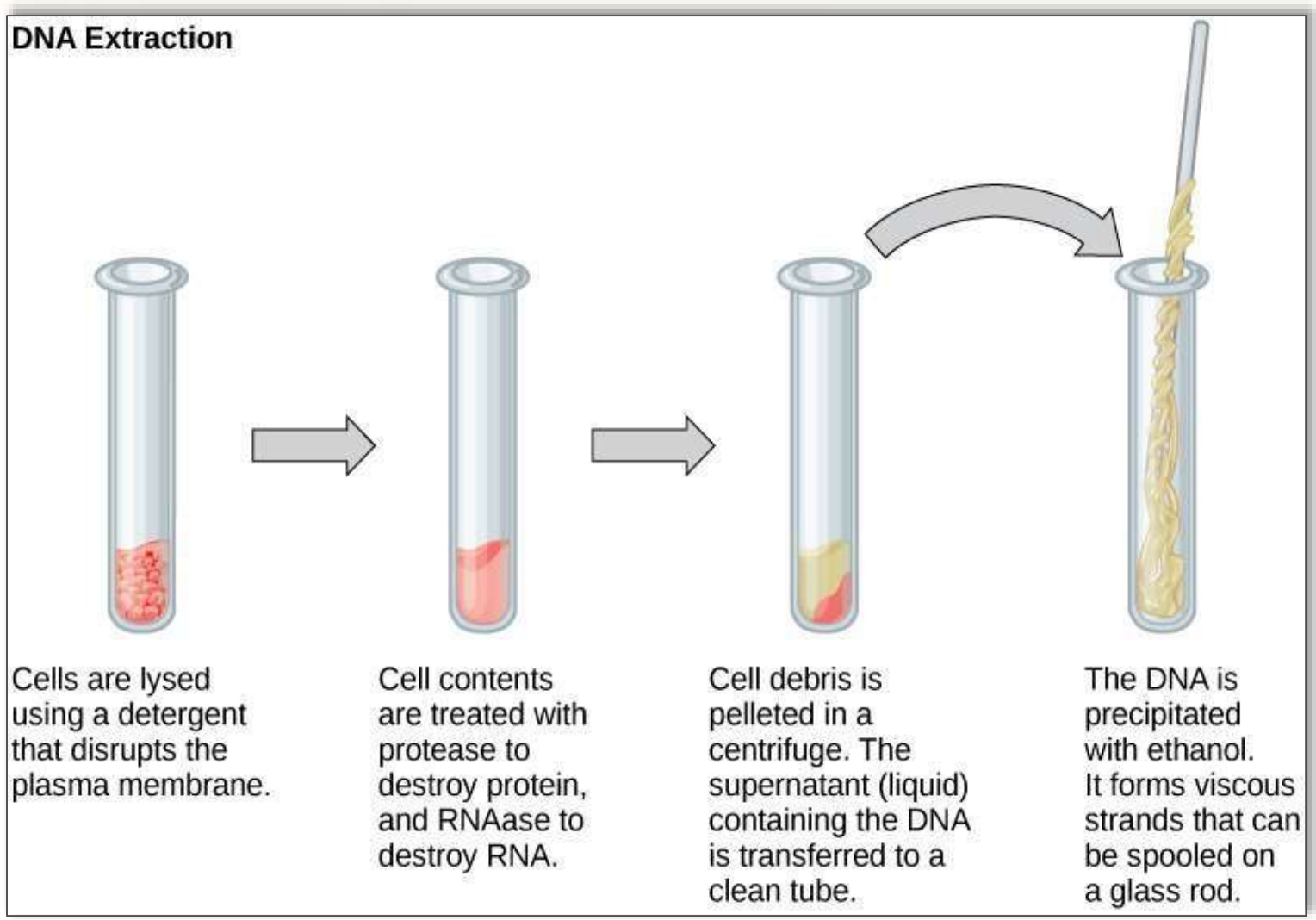
4. Multiplication and selection of clones containing the recombinant molecules

5. Expression of the gene to produce the desired product

Goals of recombinant DNA technology

- ✓ To isolate and characterize a gene
- ✓ To make desired alterations in one or more isolated genes
- ✓ To return altered genes to living cells
- ✓ Artificially synthesize new gene
- ✓ Alternating the genome of an organism
- ✓ Understanding the hereditary diseases and their cure
- ✓ Improving human genome

Isolating of DNA

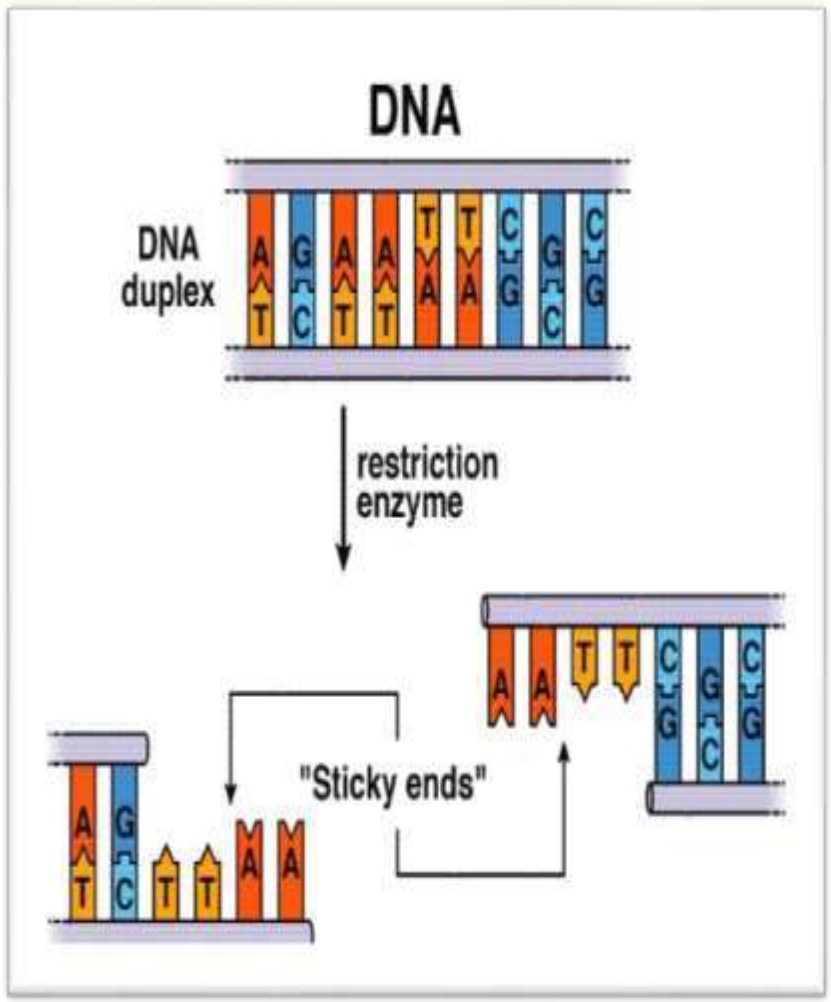


Cutting of DNA

- DNA can be cut into large fragments by mechanical shearing.

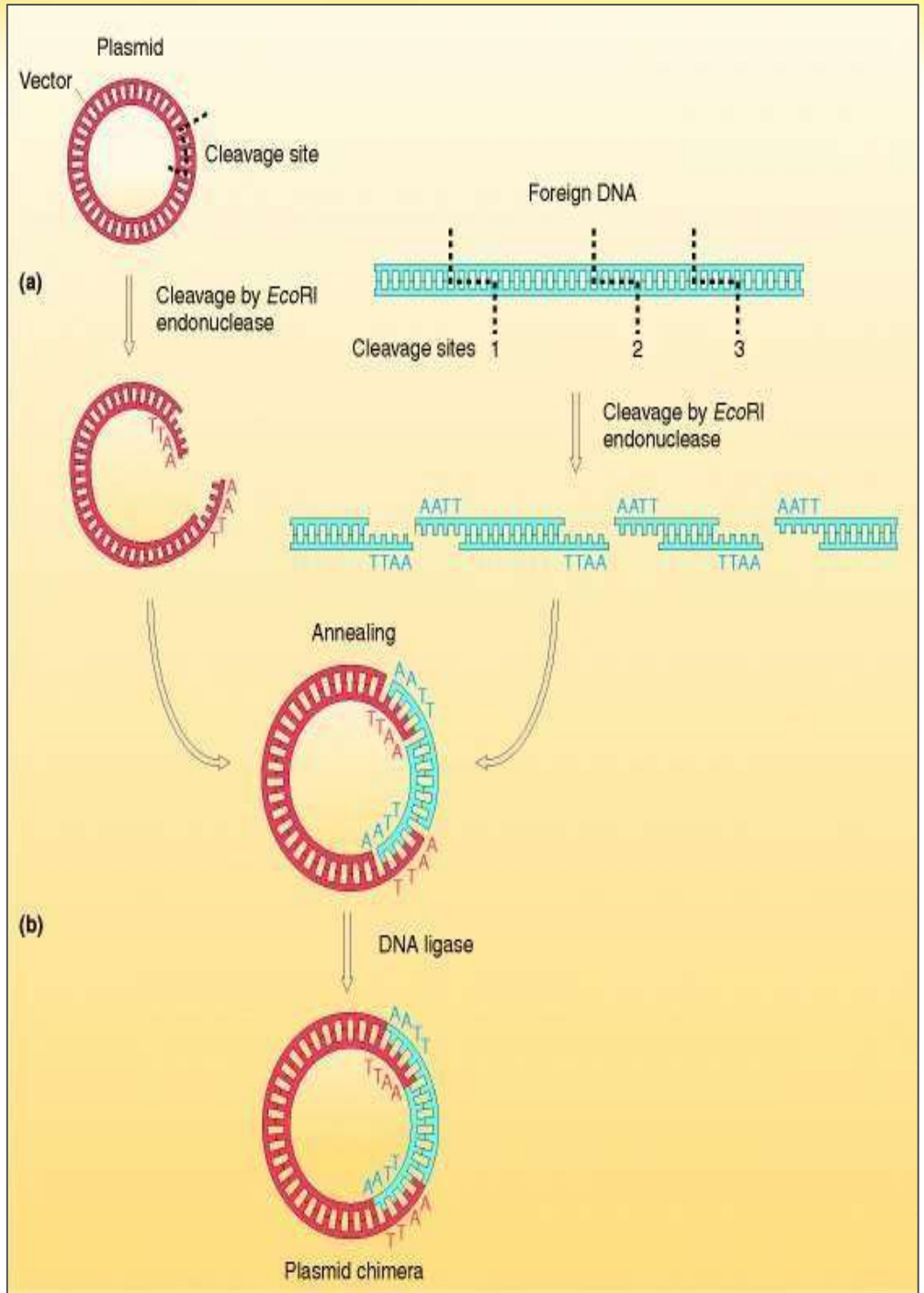
- Restriction enzymes are the scissors of molecular genetics.

Restriction enzyme



- Aspecial class of sequence-specific enzyme
- Found in bacteria
- Site-specific-cleave DNA molecules only at specific nucleotide sequence
- REases recognize DNA base sequence that are palindrome
- REase make staggered cuts with complementary base sequences for easy circulization

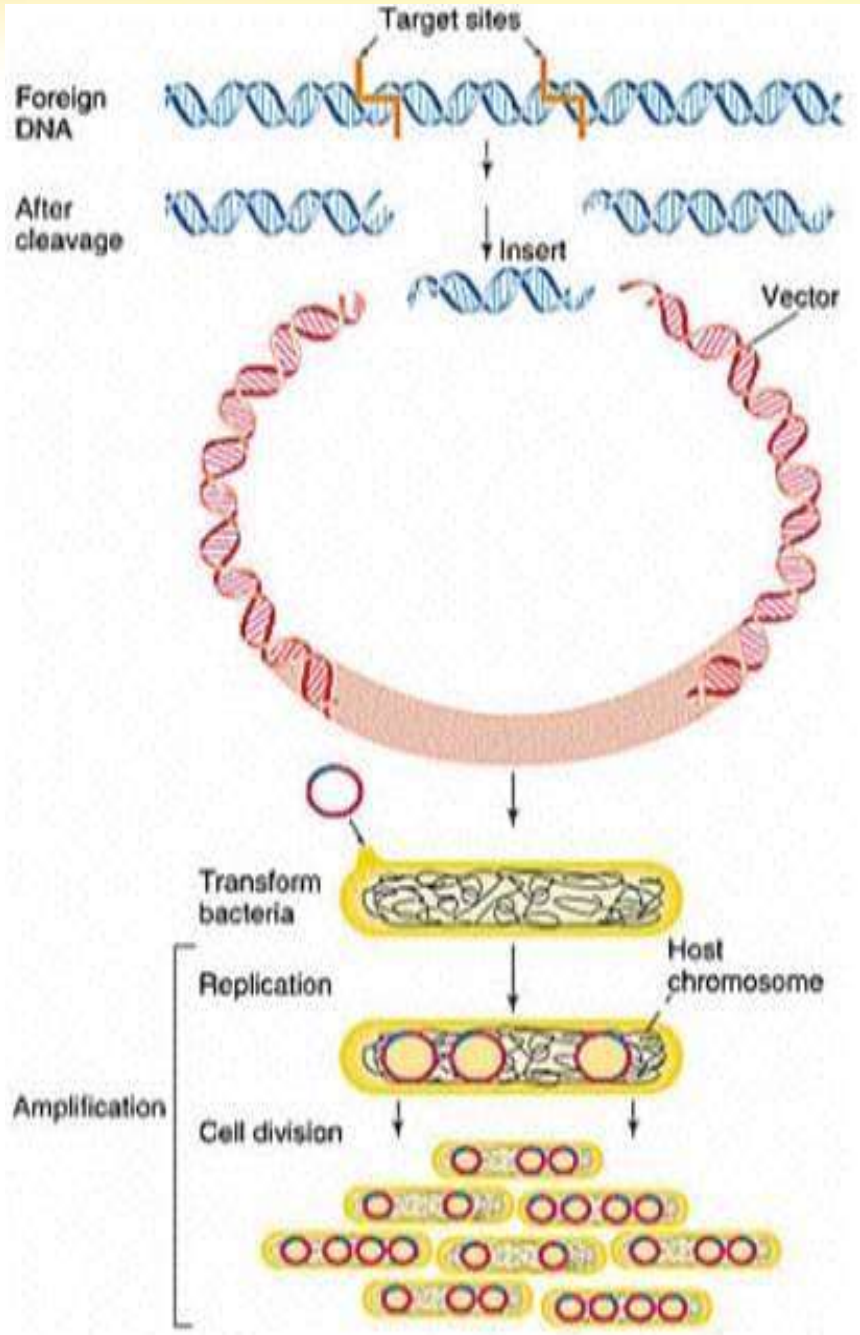
Joining DNA


Plasmid
Vector
Cleavage site
Foreign DNA
(a)
Cleavage by EcoRI endonuclease
Cleavage sites 1 2 3
Cleavage by EcoRI endonuclease
AATT
TTAA
Annealing
(b)
DNA ligase
Plasmid chimera

Amplifying the recombinant DNA

- Transforming the recombinant DNA into a bacterial host strain.
- The cells are treated with CaCl2
- DNA is added
- Cells are heat shocked at 42 C
- DNA goes into cell by a somewhat unknown mechanism.
- Once in a cell, the recombinant DNA will be replicated.
- When the cell divides, the replicated recombinant molecules go to both daughter cells which themselves will divide later.
- Thus, the DNA is amplified

Amplifying the recombinant DNA



Enzymes used inrecombinant DNA technology

DNA ligase	• Bind to DNA molecules
Type II restriction endonuclease	• Cleaves DNA at specific sites
Reverse transcriptase	• Make a DNA copy of RNA molecule
DNA polymerase I	• Fill single stranded gapes of DNA duplex
Polynycleotide Kinase	• Adds a phosephate to the 5'-OH end of a polynucleotide
Terminal transferase	• Adds homopolymer tails to the 3'-OH ends
Exonuclease III	• Removes nucleotide residues from the 3' ends
Bacteriophage {lamda} exonuclease	• removes nucleotides from the 5' ends
Alkaline phosphatase	• Removes terminal phosphates

Vectors used in rDNA technology

- A vector is an area of DNA that can join another DNA part without losing the limit for self-replication

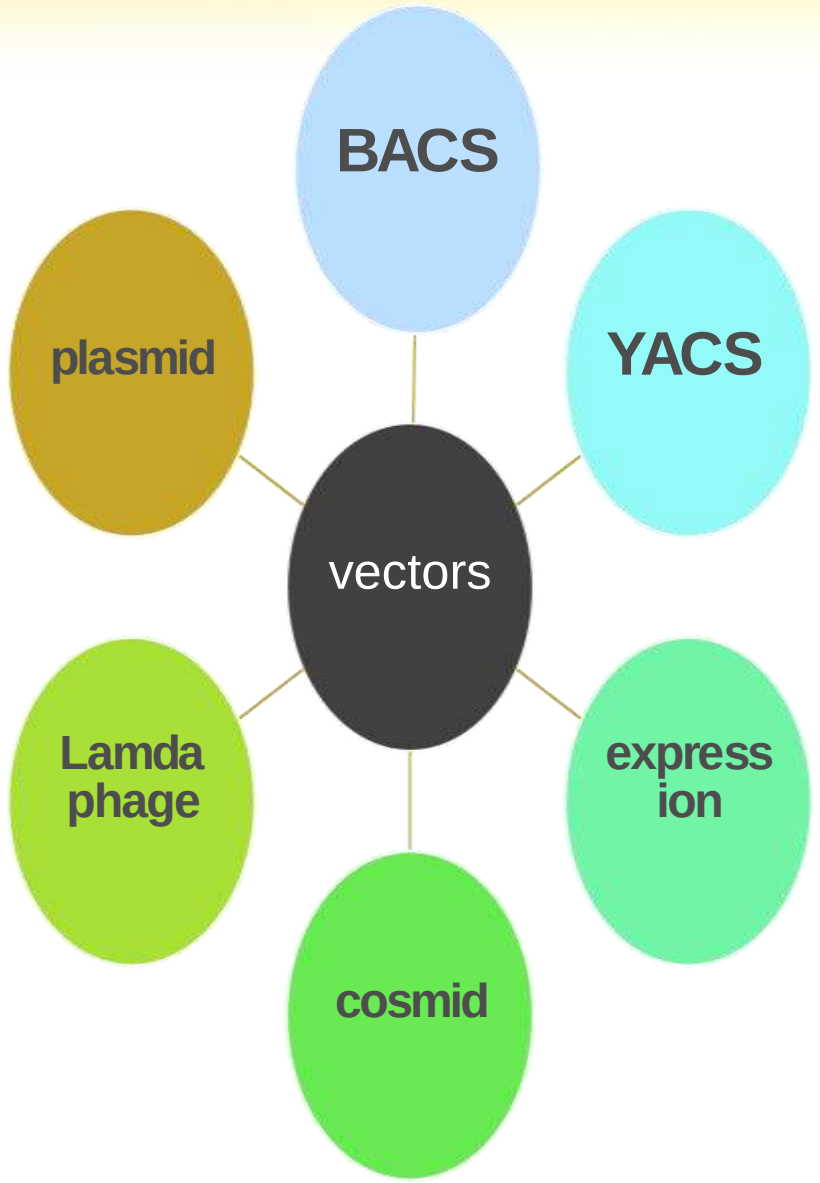
- Should be capable of replicating in host cell

- Should have convenient RE sites for inserting DNA of interest

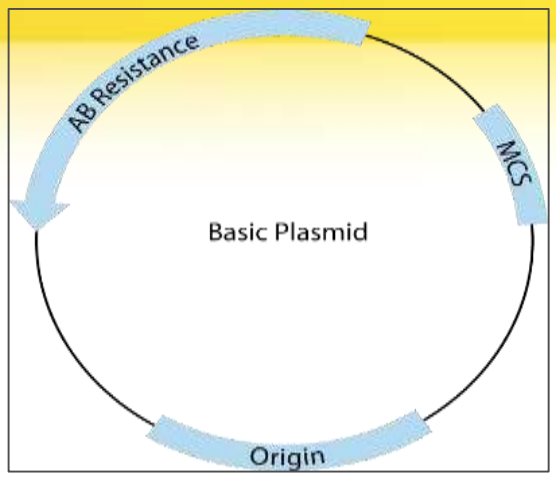
- Should have a selectable marker to indicate which host cells received recombinant DNA molecule

- Should be small and easy to isolate

Vectors used in rDNA technology

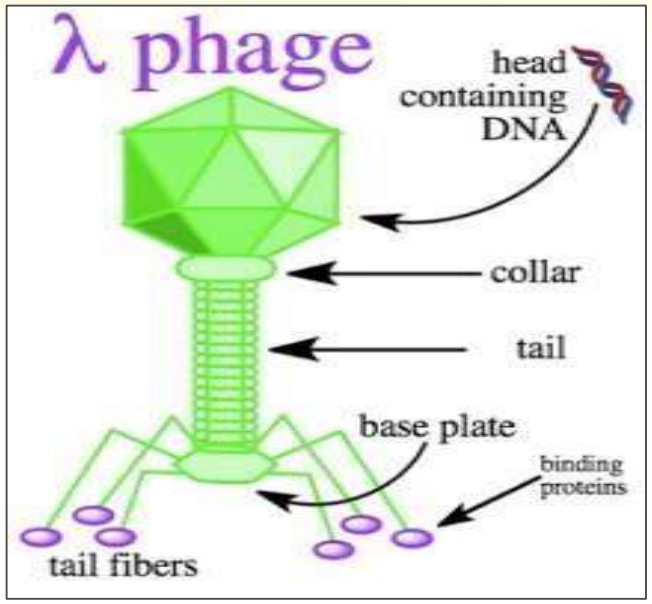


Plasmid vector



- Plasmids are small, circular DNA molecules that are separate from the rest of the chromosome.
- They replicate independently of the bacterial chromosome.
- Useful for cloning DNA inserts less that 20 kb (kilobase pairs).
- Inserts larger than 20 kb are lost easily in the bacterial cell.

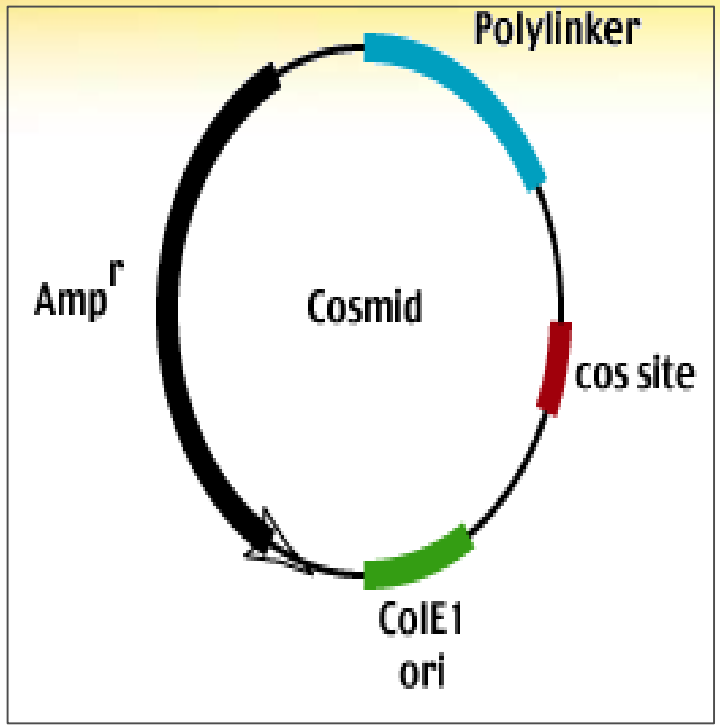
Lamda phage vector



- Lamda phage vectors are recombinant infections, containing the phage chromosome in addition to embedded "outside" DNA.

- All in all, phage vectors can convey bigger DNA groupings than plasmid vectors.

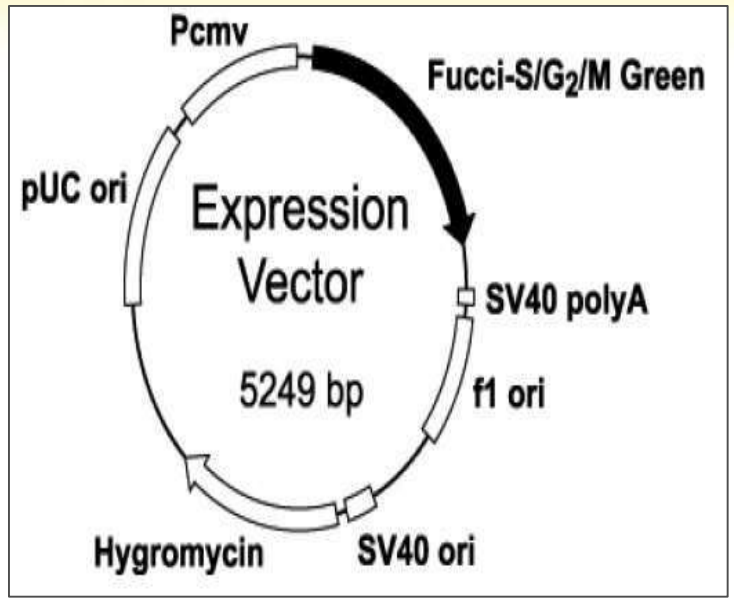
Cosmid vector



- Cosmids are hybrids of phages and plasmids that can carry DNA fragments up to 45 kb.

- They can replicate like plasmids but can be packaged like phage lambda

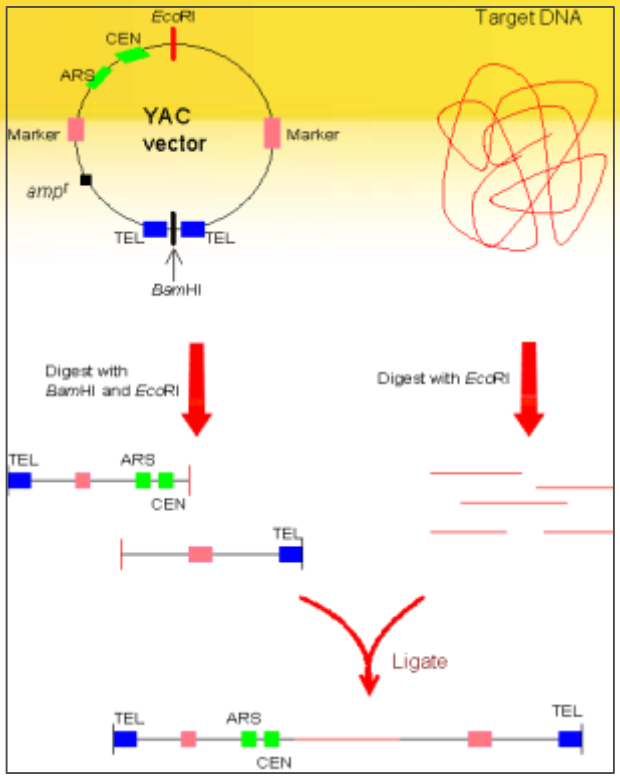
Expression vectors



- Expression vectors are vectors that carry host signals that facilitate the transcription and translation of an inserted gene.

- They are very useful for expressing eukaryotic genes in bacteria.

Yeast Artificial Chromosomes (YACS)



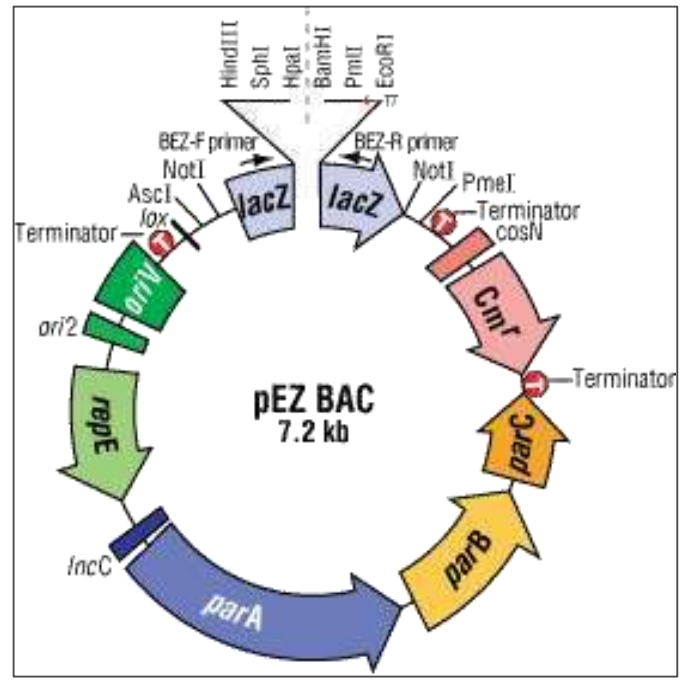
- Yeast artificial chromosomes (YACS) are yeast vectors that have been engineered to contain a centromere, telomere, origin of replication, and a selectable marker.

- They can carry up to 1,000 kb of DNA.

- they are useful for cloning eukaryotic genes that contain introns.

Bacterial Artificial Chromosomes (BACs)

- Bacterial artificial chromosomes (BACS) are bacterial plasmids derived from the F plasmid. They are capable of carrying up to 300 kb of DNA.



Methods of Gene transfer

1.Transformation

- The mechanism of the transformation process is not fully understood.

- It is believed that the $CaCl_2$ affects the cell wall, breaks at localized regions, and is also responsible for binding of DNA to cell surface.

- A brief heat shock (i.e. the sudden increase in temperature from 5°C to 40°C) stimulates DNA uptake. In general, large-sized DNAs are less efficient in transforming

2.Conjugation

- A natural microbial recombination process.
- During conjugation, two live bacteria (a donor &a recipient) come together, join by cytoplasmic bridges &transfer single stranded DNA (from donor to recipient).
- In side recipient cell, new DNA may integrate with the chromosome or may remain free.

3. Electroporation

➢Electroporation is based on the principle that high voltage electric pulses can induce cell plasma membranes to fuse.

➢Thus, electroporation is a technique involving electric field-mediated membrane permeabilization. Electric shocks can also induce cellular uptake of exogenous DNA (believed to be via the pores formed by electric pulses) from the suspending solution.

➢Electroporation is a simple and rapid technique for introducing genes into the cells from various organisms (microorganisms, plants and animals).

➢The basic technique of electroporation for transferring genes into mammalian cells is depicted in Fig. 6.11.

➢The cells are placed in a solution containing DNA and subjected to electrical shocks to cause holes in the membranes.

➢The foreign DNA fragments enter through the holes into the cytoplasm and then to nucleus.

➢Electroporation is an effective way to transform E.coli cells containing plasmids with insert DNAs longer than 100 kb. The transformation efficiency is around 109 transformants per microgram of DNA for small plasmids (about 3kb) and about 106 for large plasmids

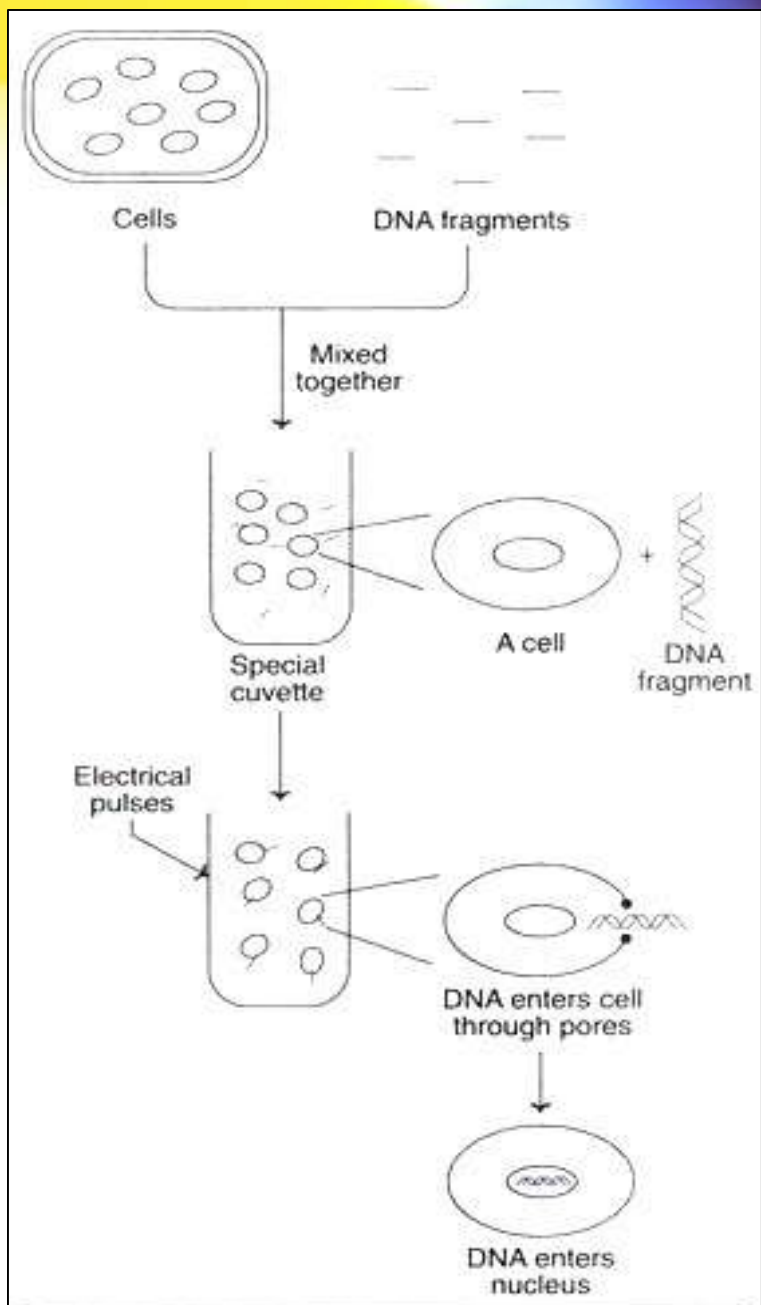


Fig. 6.11 : Gene transfer by electroporation.
(Note : Magnification depicted on right side)

4. Direct transfer of DNA

DNA is directly transferred into the nucleus by microinjection & particle bombardment.

5. Liposome -mediated gene transfer

- Liposomes are circular lipid molecules, which have an aqueous interior that can carry nucleic acids.
- Several techniques have been developed to encapsulate DNA in liposomes.
- The liposome mediated gene transfer is referred to as lipofection.
- Treatment of DNA fragment with liposomes, DNA pieces get encapsulated inside liposomes.
- These liposomes can adhere to cell membranes &fuse with them to transfer DNA fragments.The DNA enters the cell &to the nucleus.
- Positively charged liposomes efficiently complex with DNA, bind to cells &transfer DNA

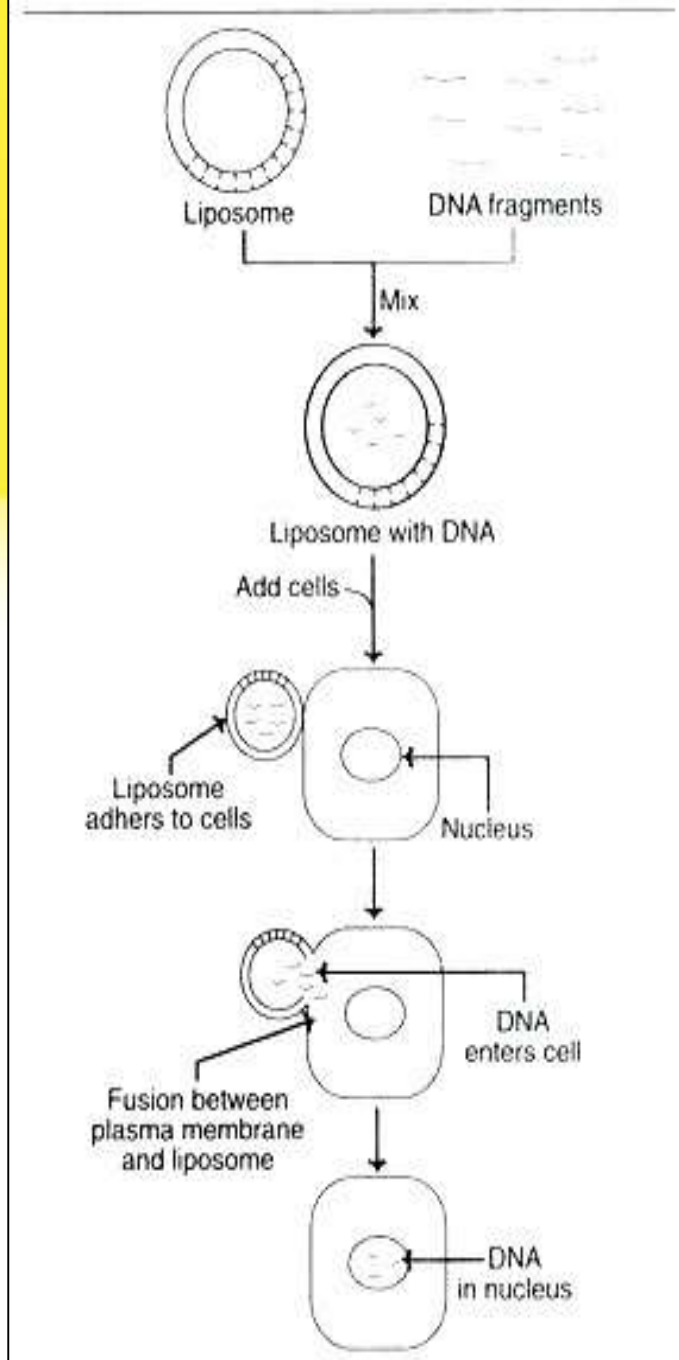


Fig. 6.12 : Liposome-mediated gene transfer
*(**Note** : For clarity, the native cell DNA is not shown).*

Host cells

The hosts are the living systems or cells in which the carrier of recombinant DNA molecule or vector can be propagated.
There are different types of host cells prokaryotic and eukaryotic .

Group	Examples
Prokaryotic	
Bacteria	E.Coli Bacillus subtilis Streptomyces sp.
Eukaryotic	
Fungi	Saccharomyces cerevisiae Aspargilus nidulans
Animals	Insect cells Oocytes Mammalians cells
Plants	Protoplasts Intact cells

Why genetic engineering is done?

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- **Genetic engineering** is when the **genetic** makeup of an organism is altered by inserting, deleting or changing specific pieces of DNA. When conducting **genetic engineering**, the organisms that have their **genetic** makeup altered are referred to as **genetically** modified organisms, or GMOs.

Applications

- Molecular Biology: Gene Mapping
- Genetic Disorder
- Monoclonal Ab product
- Gene Therapy
- DNA Fingerprinting
- Vaccines
- Pharma Products

Gene Mapping

- **Genetic mapping** - also called linkage **mapping** - can offer firm evidence that a disease transmitted from parent to child is linked to one or more **genes**. **Mapping** also provides clues about which chromosome contains the **gene** and precisely where the **gene** lies on that chromosome.

- **Chromosome mapping** is a technique used in autosomal DNA testing which allows to determine which segments of DNA came from which ancestor. In order to **map** DNA segments on specific **chromosomes** it is necessary to test a number of close family relatives.

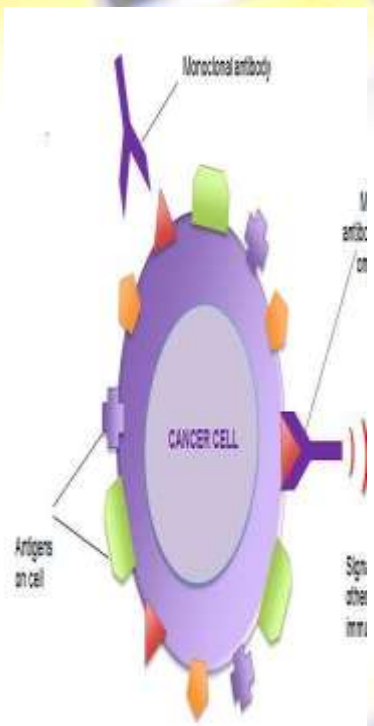
Genetic Disorder

- A **genetic disorder** is a **disease** that is caused by an abnormality in an individual's DNA.

- Abnormalities can be as small as a single-base mutation in just one **gene**, or they can involve the addition or subtraction of entire chromosomes.

- **Most common disorders:** Cystic Fibrosis, Down syndrome

Monoclonal Ab production



- Hybridoma technique has made it possible to produce monoclonal antibodies.
- In this technique, the lymphocytes or B cells are joined with myeloma cells; the resulting substance is called as Hybridoma.
- This hybridoma produces unlimited antibodies in culture. T
- he antibodies produced are called monoclonal antibodies. These antibodies are used to produce vaccines against different viral infections.
- **Monoclonal antibodies** are **used** in a wide variety of ways. They are **used** in pregnancy test kits to identify the small levels of a hormone called human chorionic gonadotrophin, which is present in the urine of pregnant women.
- They can also be **used** to locate blood clots as they bind to clots.

DNA Fingerprinting

- Medical **Definition** of **DNA fingerprinting**.: a technique used especially for identification (as for forensic purposes) by extracting and identifying the base-pair pattern of an individual's **DNA**—called also **DNA** typing, genetic **fingerprinting**.

- **DNA fingerprinting**. Image caption:
- In **DNA fingerprinting**, scientists collect **samples of DNA** from different sources — for **example**, from a hair left behind at the crime scene and from the blood of victims and suspects.

- They then narrow in on the stretches of repetitive **DNA** scattered throughout these **samples**.

Vaccines

- **A vaccination** is the injection of **a killed or weakened organism** that produces immunity in the body against that organism.
- An antigen is a chemical substance that will trigger an immune response in the human body and this will cause the body to **produce** antibodies.
- Usually virus proteins or a weakened virus are used as **vaccine** antigens.
- Recombinant DNA technology has made it easier for scientists to develop vaccines by cloning the gene used for protective antigen protein. Viral vaccines are mostly developed from this technique, for example Herpes, Influenza, Hepatitis, Foot and Mouth disease.

Vaccines

Recombinant vaccines can be broadly grouped into two kinds:

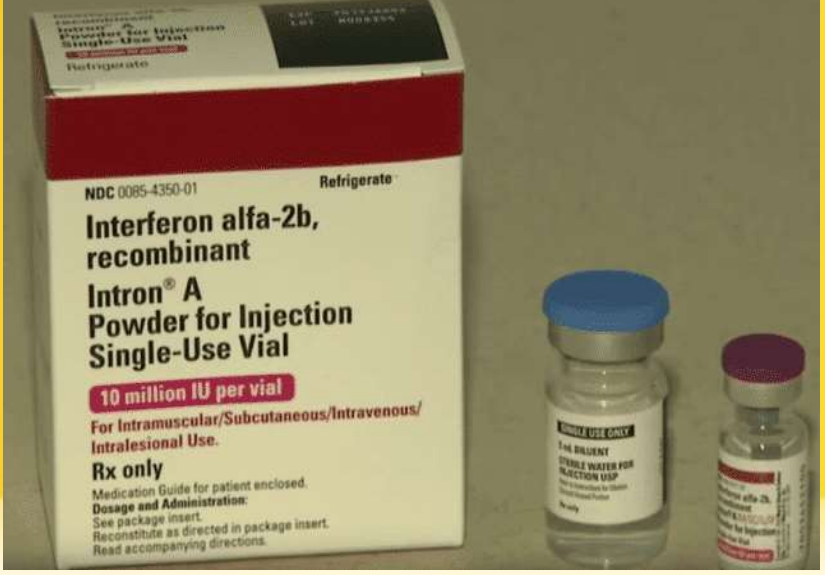
(i) **Recombinant protein vaccines**: This is based on production of recombinant DNA which is expressed to release the specific protein used in vaccine preparation

(ii) **DNA vaccines:** Here the gene encoding for immunogenic protein is isolated and used to produce recombinant DNA which acts as vaccine to be injected into the individual.

Pharma Products

- A pharmaceutical drug is a drug **used to diagnose, cure, treat, or prevent disease.**

a. Antibiotics can be produced by microorganisms as well as in the laboratory. Alexander Fleming discovered Penicillin in 1928 using recombinant DNA technology. Other biotechnological techniques are also being used to produce antibiotics.



Pharma Products

b. Interferon

- A glycoprotein that has the ability to block the multiplication or division of viruses in the cells or nearby cells are called interferons.
- It can be used to treat cancer like hairy cell leukemia. Recombinant DNA technology produces this protein using E.coli.
- Interferon alpha is used to treat lymphoma and myelogenous leukemia.

Interferon formulations in Market

Generic name	Trade name
Interferon alpha 2a	Roferon A
Interferon alpha 2b	Intron A/Reliferon/Uniferon
Human leukocyte Interferon-alpha (HulFN-alpha-Le)	Multiferon
Interferon beta 1a, liquid form	Rebif
Interferon beta 1a, lyophilized	Avonex
Interferon beta 1a, biogeneric (Iran)	Cinnovex
Interferon beta 1b	Betaseron/ Betaferon
Interferon gamma 1b	Actimmune
PEGylated interferon alpha 2a	Pegasys
PEGylated interferon alpha 2a(Egypt)	Reiferon Retard
PEGylated interferon alpha 2b	PegIntron

Pharma Products

c. Human Insulin

- Recombinant DNA technology has allowed the scientists to develop human insulin by using the bacteria as a host cell.

- A variety of different recombinant insulinmpreparations are in widespread use. Recombinant insulin isnsynthesized byinserting the human insulin gene into E. coli,which then produces insulin for human use.

- This is supposed to be safer than traditionally prepared drugs

d. Human Growth Hormones

- Human growth hormone is a polypeptide hormone. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers.

- This unsafe practice led to some patients developing Creutzfeldt Jacob disease. Recombinant HGH eliminated this problem, and is now used therapeutically.

- It has also been misused as a performance enhancing drug by athletes and others. In recent days biotechnology has helped scientists to produce many growth hormones.

- The dwarfism disease is successfully treated with this hormone.

e. Diagnosis of infection with HIV:

- Each of the three widely used methods for diagnosing HIV infection has been developed using recombinant DNA.

- The antibody test (ELISA or western blot) uses a recombinant HIV protein to test for the presence of antibodies that the body has produced in response to an HIV infection.

- The DNA test looks for the presence of HIV genetic material using reverse transcriptase polymerase chain reaction (RTPCR).

- Development of the RT-PCR test was made possible by the molecular cloning and sequence analysis of HIV genomes.

THERAPEUTIC AGENTS FOR HUMAN DISEASES

DNA Product	Trade name	Application / Uses
Insulin	Humulin	Diabetes
Growth hormone	Protropin/Humatrope	Pituitary dwarfism
Interferon	Intron A	Hairy cell leukemia
Hepatitis B vaccine	Recombinax HB/ Engerix	Hepatitis B
TissuEplasminogen activator	Activase	Myocardial infarction
Factor vIII	Kogenate/Recombinate	Hemophilia
Dnase	Pulmozyme	Cystic fibrosis
Erythropoietin	Epogen/rocrit	Severe anemia with kidney damage

Thank You