Application of r DNA technology and genetic engineering in the production of: i)Interferon ii) Vaccines- hepatitis- B iii) Hormones-Insulin.

Introduction

- **Genetic engineering** has provided a way to create new pharmaceutical **products** called recombinant DNA pharmaceuticals. Such **products** include antibiotic drugs, vaccines, and hormones used to treat various diseases.
- For example, the naturally occurring antibiotic synthesis pathways of various ***Streptomyces*** spp., long known for their antibiotic production capabilities, can be modified to improve yields or to create new antibiotics through the introduction of genes encoding additional enzymes.

Some Genetically Engineered Pharmaceutical Products

S.No	Recombinant DNA Product	Application
1.	Atrial natriuretic peptide	Treatment of heart disease (e.g., congestive heart failure), kidney disease, high blood pressure
2.	DNase	Treatment of viscous lung secretions in cystic fibrosis
3.	Erythropoietin	Treatment of severe anemia with kidney damage
4.	Factor VIII	Treatment of hemophilia
5.	Hepatitis B vaccine	Prevention of hepatitis B infection
6.	Human growth hormone	Treatment of growth hormone deficiency, Turner's syndrome, burns
7.	Human insulin	Treatment of diabetes
8.	Interferons	Treatment of multiple sclerosis, various cancers (e.g., melanoma), viral infections (e.g., Hepatitis B and C)
9.	Tetracenomycins	Used as antibiotics
10.	Tissue plasminogen activator	Treatment of pulmonary embolism in ischemic stroke, myocardial infarction

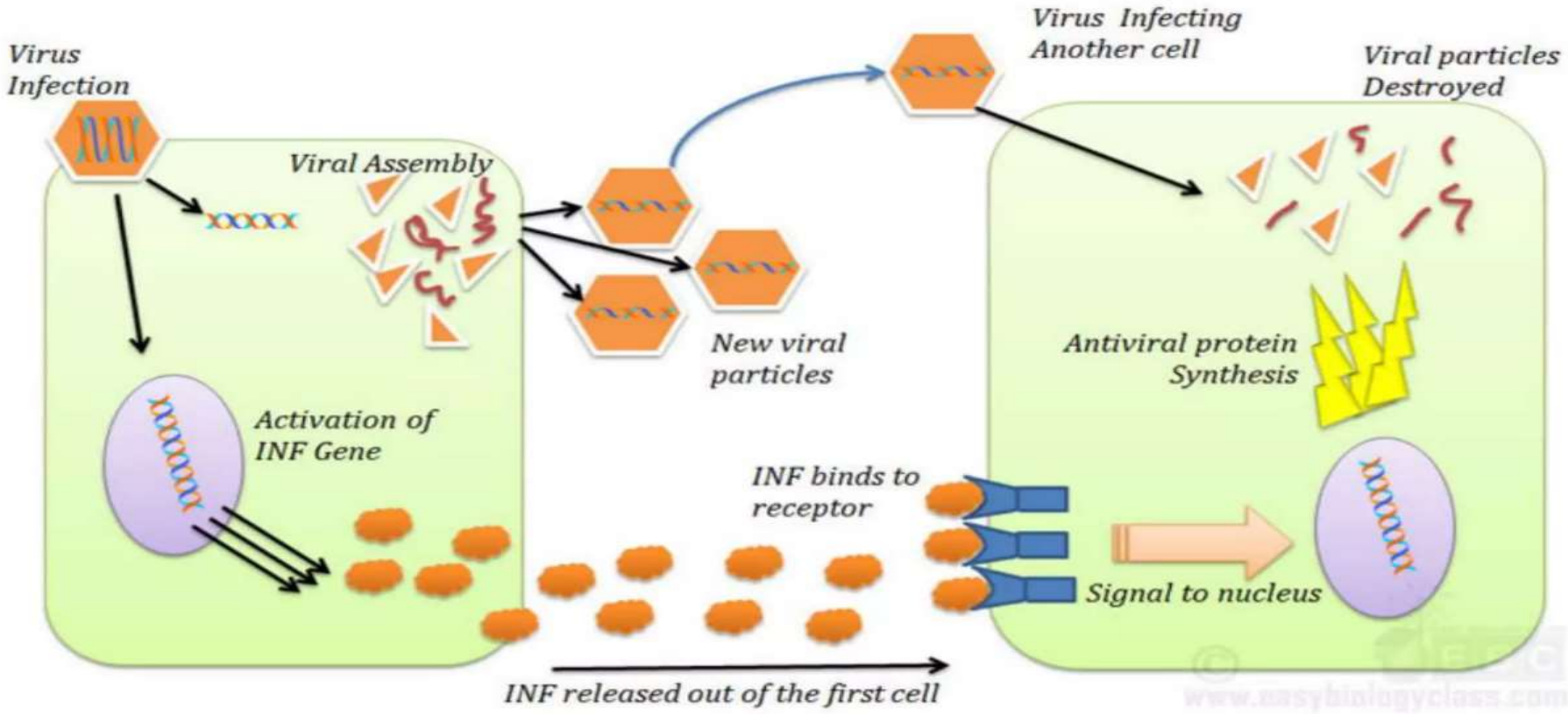
Interferon

- Interferon is the molecule which interferes with viral replication without disturbing the cellular metabolism and it may serve as antiviral agent.
- The large quantity of interferon is produced by gene cloning i.e., introducing rDNA of interferon gene into *E.coli*.
- Interferon is available in the market with trade names Intron A, Roferon, Wellferone, Shanferone.
- Interferons are the set of small proteins which are secreted by the cell in response to the viral infections.
- Its molecular weight ranges between 20,000 to 30,000 Daltons.

Interferon

- Interferons are broadly grouped into 3 types.
  - Interferon α– Leucocytes
  - Interferon β – Fibroblasts
  - Interferon γ – Lymphocytes
- Interferon does not prevent the virus from infecting the cells but inhibits its intracellular replication.
- rDNA has served as useful tool in the production of interferon.
- The nucleotide sequence of genes of INF α & INF β are obtained from leucocyte interferon and fibroblastic interferon respectively had quite similar structure.

Anti-Viral Action of Interferon



Anti-Viral Action of Interferon

- The synthesis and release of interferons from a cell is induced by the viral particles or by dsRNA of virus.
- Specific interferons are recognized by receptors present on the plasma membrane. Once a cell receives the stimuli, the interferon proteins are synthesized and release out of the cell.
- The secreted interferon molecules then bound to the **ganglioside receptors** on the plasma membrane of another cell.
- The binding of interferons to its receptors triggers the production of many enzymes that render the cell's resistance to the viral infection.
- The two important antiviral enzymes whose production is triggered by the INF are **Oligo(A) Synthetase** and **Protein Kinase R** (PKR).
- When an INF-stimulated cell is infected by the viral particle, the viral protein synthesis is inhibited by an active endoribonuclease enzyme by degrading the viral RNA.

Functions of Interferons in the Cell

- Indirect antiviral properties
- Restrict the replication and assembly of viral particles
- Interferons can activate T cells
- Interferons can activate macrophages, neutrophils and natural killer cells.
- Enhance the phagocytic property of macrophages.
- Increase the antigen presenting capacity of antigen presenting cells.

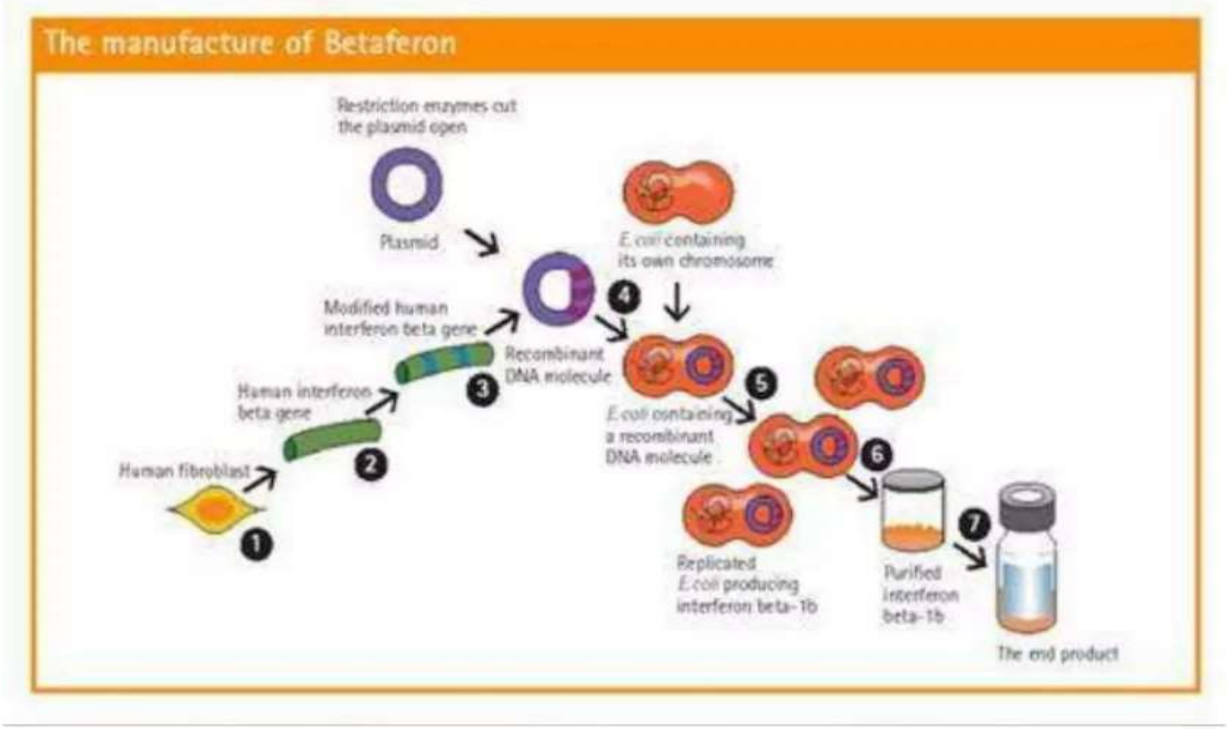
Applications of Interferons

- INFα is used in the treatment of Hepatitis B and C infections
- INFβ is used in the treatment of multiple sclerosis and autoimmune disorders.
- Interferons have antitumor properties
- Combined therapy of interferon and chemotherapy is used in the treatment of some cancers.

Production of Interferons

- The synthesis of INF based on molecular biology was successful when DNA sequence coding for human leucocyte INF was attached to the yeasts alcohol dehydrogenase gene in a plasmid and was introduced into cells of *Saccharomyces cerevisiae*.
- The yeast cells could synthesize about one million molecules of INF.
- In E.coli the plasmid could also successfully replicate, but production is relatively slow in *E.coli* because in yeast it is easy to grow and replicate glycoprotein derived from mammalian cells.

Production of Interferons



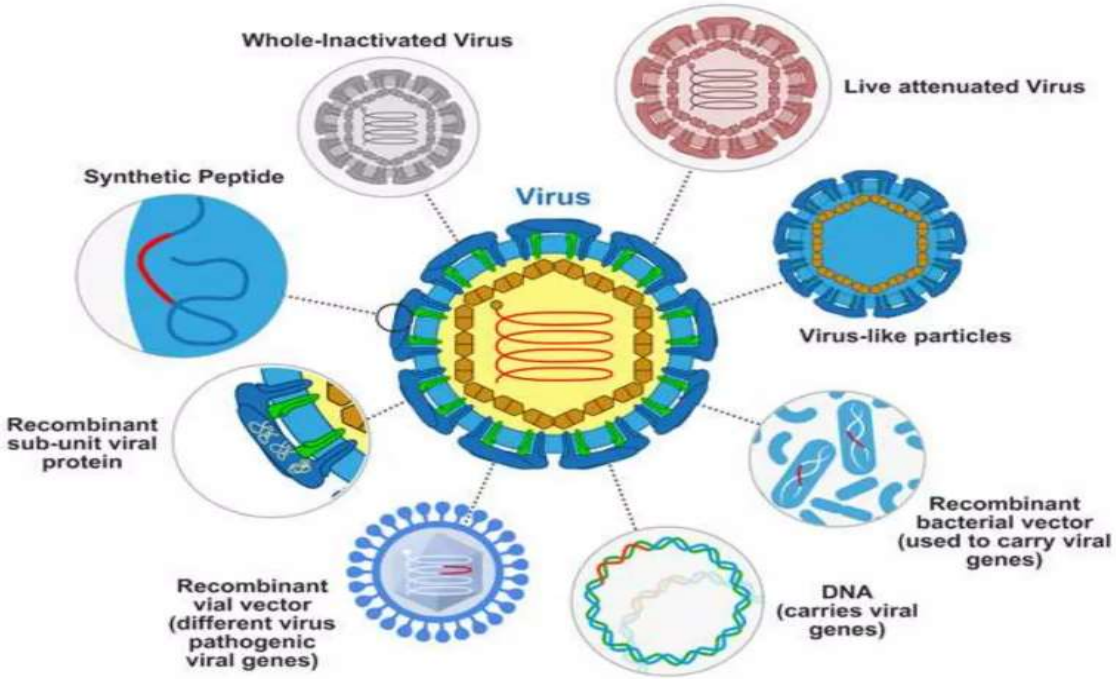
Hepatitis- B Vaccine

- Hepatitis B virus (HBV) is one of the most common infectious diseases known to man.
- The World Health Organization (WHO) estimates that there are as many as 285 million chronic carriers of this virus worldwide.
- Hepatitis B is 50 to 100 times more infectious than AIDS.
- Hepatitis B is irritation and swelling (inflammation) of the liver due to infection with the hepatitis B virus (HBV).
- Other types of viral hepatitis include: Hepatitis A, Hepatitis C, Hepatitis D.
- It produces several chronic liver disorders such as Fulminant chronic hepatitis, cirrhosis and primary liver cancer.

Hepatitis-B Vaccine

- HB virus has been identified as a 42-nm particle containing a double stranded circular DNA molecule of about 3Kb size.
- DNA genome has a relative molecular mass of approximately 2×10^6
- DNA cloning. It's an unusual feature among other viruses.
- Plasma of human has been detected to have varying amount of HB antigens. Three types of viral coat proteins are recognized to be antigenic -
  - viral surface antigen (HBsAg)
  - viral core antigen (HBcAg)
  - the e-antigen (HBeAg)

Hepatitis-B Vaccine



Hepatitis-B Vaccine

- In 1986, the Recombivax HB vaccine for hepatitis B was approved for human use in several countries, the culmination of research started by William Rutter, Pablo Valenzuela and colleagues in 1979 on the cloning of hepatitis B virus (HBV) antigens.
- It was the first vaccine to be produced using recombinant DNA technology and although it was only the third recombinant product to be approved for clinical use.

Hepatitis-B Vaccine

- Surface antigen HBsAg is found as 18-22 nm spherical or tubular form particles. Recently HBsAg gene or it's subunits are used for the production of recombinant Hepatitis B vaccine.
- After infection in human being, HBV fails to multiply and infect a large number of cells and even does not grow in cultured cells.
- Rather, HB viral antigen is obtained from the plasma of infected persons. This property has been explained to be due to hindrance of its molecular characterization and expression, and thus helps in the development of vaccines.

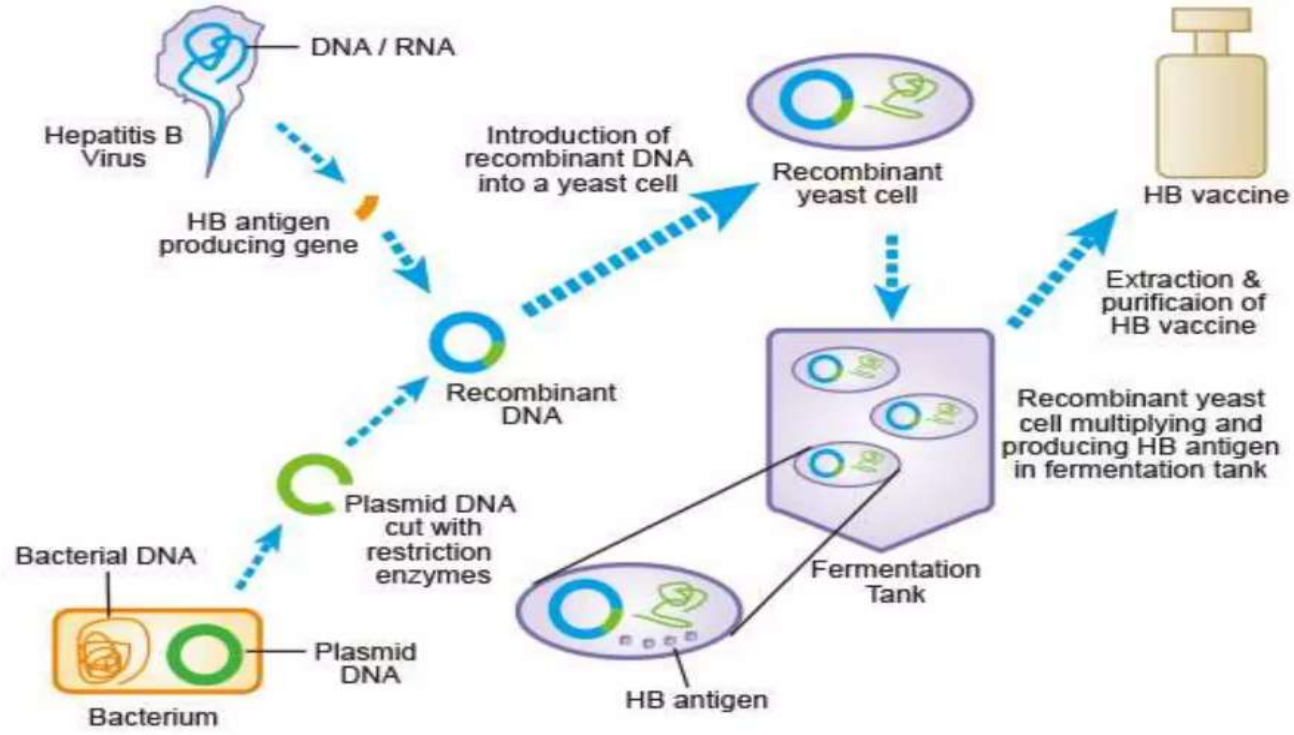
Steps Involved in Production of Hepatitis-B Vaccine

1. HBs antigen producing gene is isolated from the HB virus by normal isolation process (cell lysis, protein denaturation, precipitation, centrifugation and drying).
2. A plasmid DNA is extracted from a bacterium- *E.coli* and is cut with restriction enzyme- Eco RI forming the plasmid vector.
3. The isolated HBs antigen producing gene is located and inserted into the bacterial plasmid vector on forming the recombinant DNA.
4. This recombinant DNA, containing the target gene, is introduced into a yeast cell forming the recombinant yeast cell.

Steps Involved in Production of Hepatitis-B Vaccine

5. The recombinant yeast cell multiplies in the fermentation tank and produces the HBs antigens.
6. After 48 hours, yeast cells are ruptured to free HBsAg. The mixture is processed for extraction.
7. The HBs antigens are purified.
8. HBsAg are combined with preserving agent and other ingredients and bottled. Now it is ready for vaccination in humans.

Hepatitis-B Vaccine



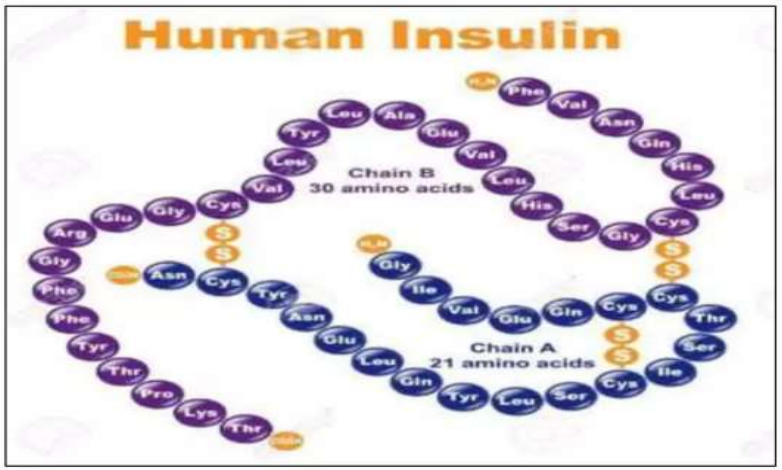
Human Insulin

- The earliest uses of biotechnology in pharmaceutical manufacturing is the use of recombinant DNA technology to modify *Escherichia coli* bacteria to produce human insulin, which was performed in 1978 at Genentech.
- **Insulin** is a hormone produced by β-cells of islets of Langerhans of pancreas. It was discovered by sir Edward Sharpey Schafer (1916) while studying Islets of Langerhans
- Insulin is a peptide hormone produced by pancreas and is a central regulator of carbohydrates and fat metabolism in the body.

Structure of Human Insulin

- Chemically Human insulin is small, simple protein composed of 51 amino acids sequences and has a molecular weight of 5808 Da.
- Insulin hormone is a dimer of a A- chain and a B-chain which are linked together by a disulphide bond.

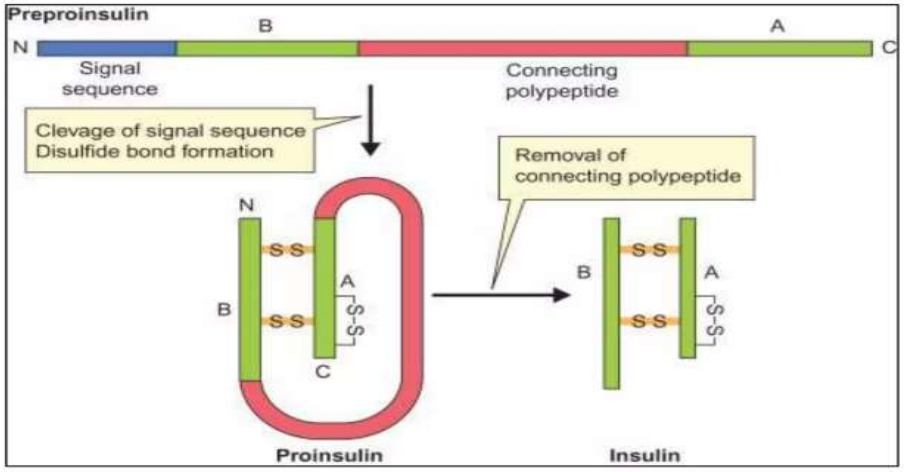
- Fredrick Sanger et al (1954) gave the first complete description of insulin. Insulin consists of two polypeptide chain,
  - o Chain A-21 amino acids long
  - o Chain B-30 amino acids long
  - o Both chains are joined together by disulphide bond between two cysteine residue



Insulin Produced Inside Pancreas

- At first Pancreatic β-cells synthesize pre-pro-insulin, which is a 109 amino acids long polypeptide
- Among 109 amino acids, 23 amino acids are signal molecules which allows the pre-pro-insulin to pass through cell membrane.

- Entering inside cell, it become 86 amino acids long pro-insulin. It is still inactive.
- Some Proteolytic enzymes cut and expose the active site of pro insulin converting it into active form of insulin of 51 amino acids long.



Production of Human Insulin

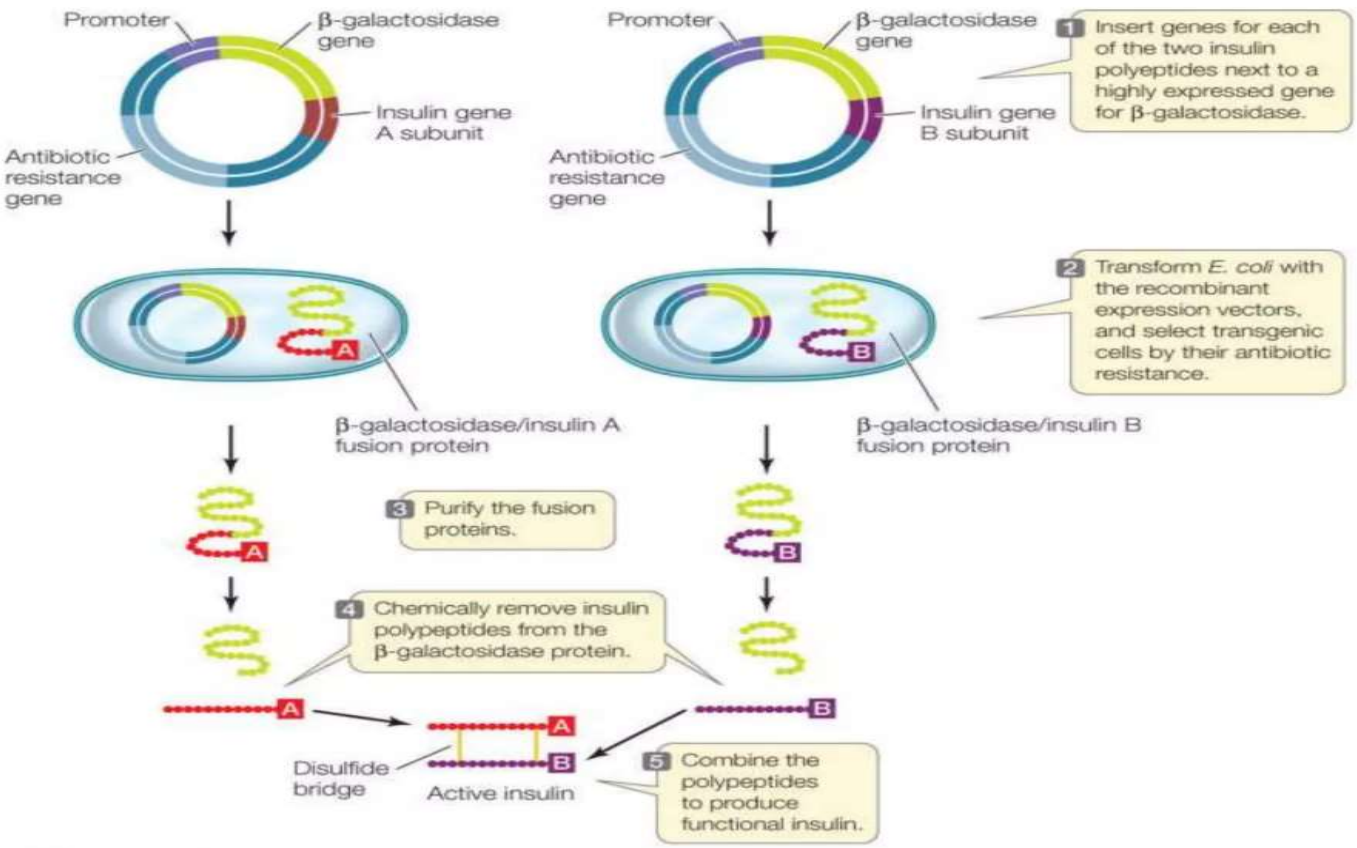
•The basic step in recombinant DNA technology is similar for insulin production also.

- At first suitable vector (plasmid) is isolated from E. coli and then it is cut open by restriction endonuclease enzyme.
- The gene of interest (ie. Insulin coding gene) is isolated from β-cell and inserted in opened plasmid.
- Plasmid and gene of interest are recombined together by DNA ligase enzyme
- This recombined plasmid is inserted into suitable host cell (ie E. coli) and now this recombined host cell starts producing insulin hormone.

Production of Human Insulin

•Hakura et al (1977) chemically synthesize DNA sequence of insulin for two chains A and B and separately inserted into two PBR322 plasmid vector.

- These gene are inserted by the side of β-galactosidase gene of the plasmid.
- The recombinant plasmid were then separately transformed into E. coli host.
- The recombinant host produced pro-insulin chains ie. fused β-galactosidase-A chain and β-galactosidase-B-chain separately.
- These pro-insulin chains A and B were separated from β-galactosidase by treatment with cyanogen bromide. The detachment of pro-insulin chains from β-galactosidase is possible because an extra codon form methionine was added at N-terminal of each gene for A and B-chain.
- After detachment, A and B chains are joined invitro to reconstitute the naïve insulin by sulphonating the peptide chains with sodium disulphonate and sodium sulphite.


Promoter
β-galactosidase gene
Insulin gene A subunit
Antibiotic resistance gene
Promoter
β-galactosidase gene
Insulin gene B subunit
Antibiotic resistance gene
1 Insert genes for each of the two insulin polyeptides next to a highly expressed gene for β-galactosidase.
2 Transform E. coli with the recombinant expression vectors, and select transgenic cells by their antibiotic resistance.
β-galactosidase/insulin A fusion protein
β-galactosidase/insulin B fusion protein
3 Purify the fusion proteins.
4 Chemically remove insulin polypeptides from the β-galactosidase protein.
A
B
Disulfide bridge
Active insulin
5 Combine the polypeptides to produce functional insulin.


Humulin N