

EVALUATION OF RECOMBINANT PROTEINS



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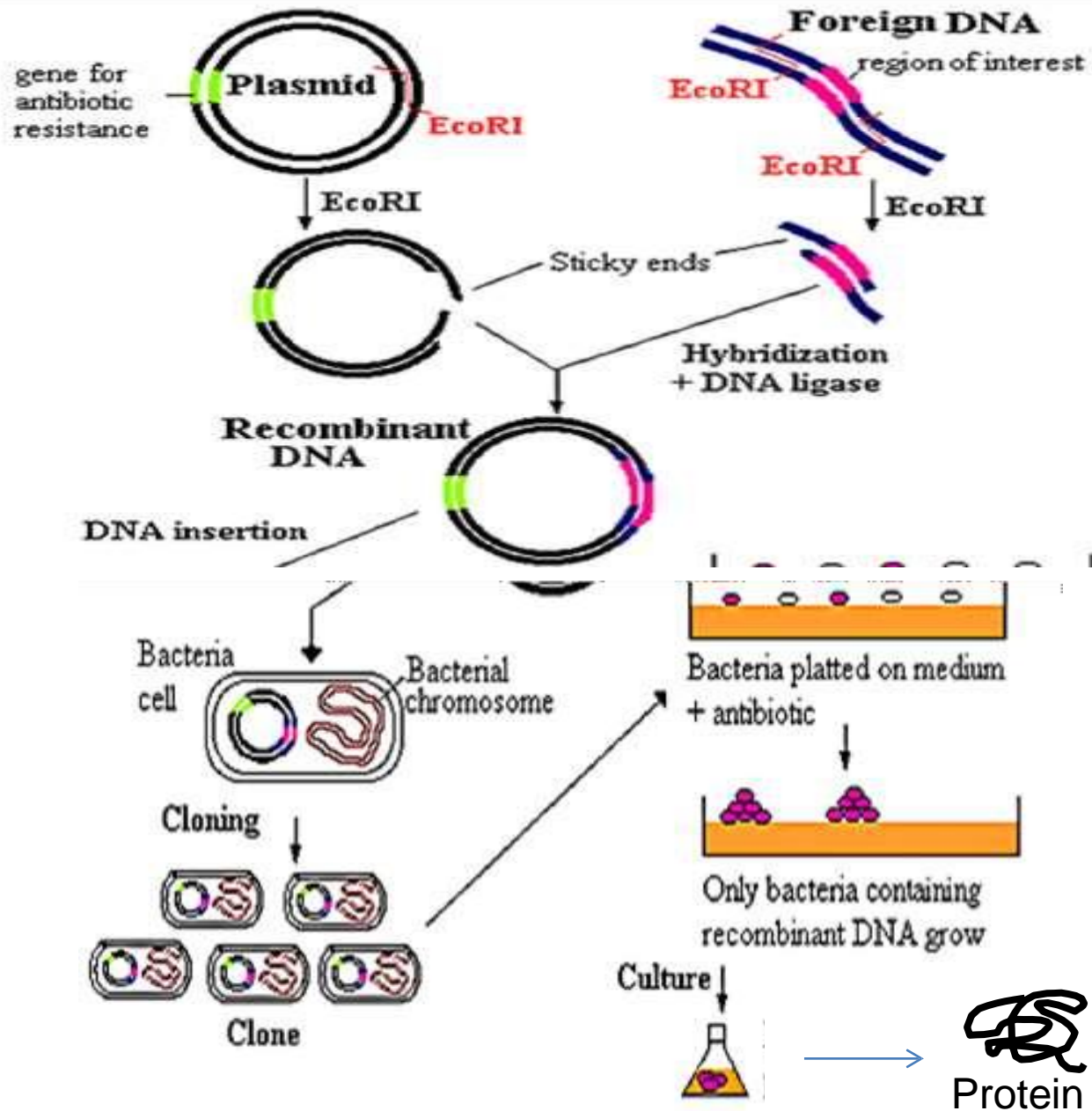


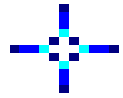
Introduction

- **Proteins** are the most abundant organic molecules of the living system. They have significant role in structural and functional organisation of the cell.
- Proteins that result from the expression of recombinant DNA within living cells are termed recombinant proteins.
- **Recombinant DNA** technology involves taking genetic material from one source and recombining it in vitro with another source followed by introducing of recombined material into host cell.
- Once a Recombinant DNA is inserted into bacteria, these bacteria will make protein based on this rDNA. This protein is know as **Recombinant Protein**.

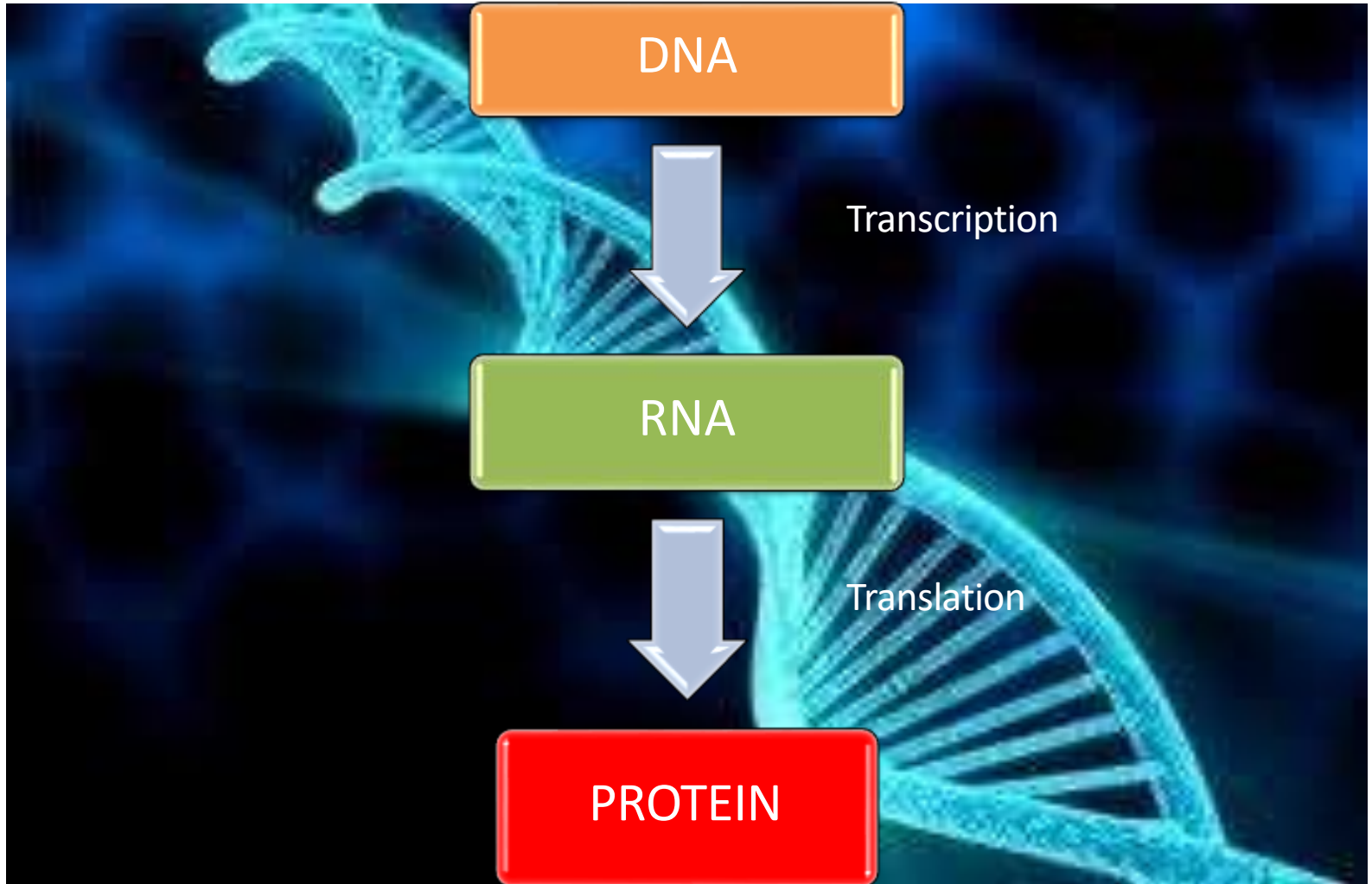


rDNA





Gene Expression



Protein Expression and Purification

- Isolation of genes.
- Insertion of isolated gene to expression vector.
- Transfer of recombinant vector into host cell through Transformation.
- Identification and isolation of cells containing recombinant vector.
- Growth of cells through fermentation.
- Isolation and purification of protein.

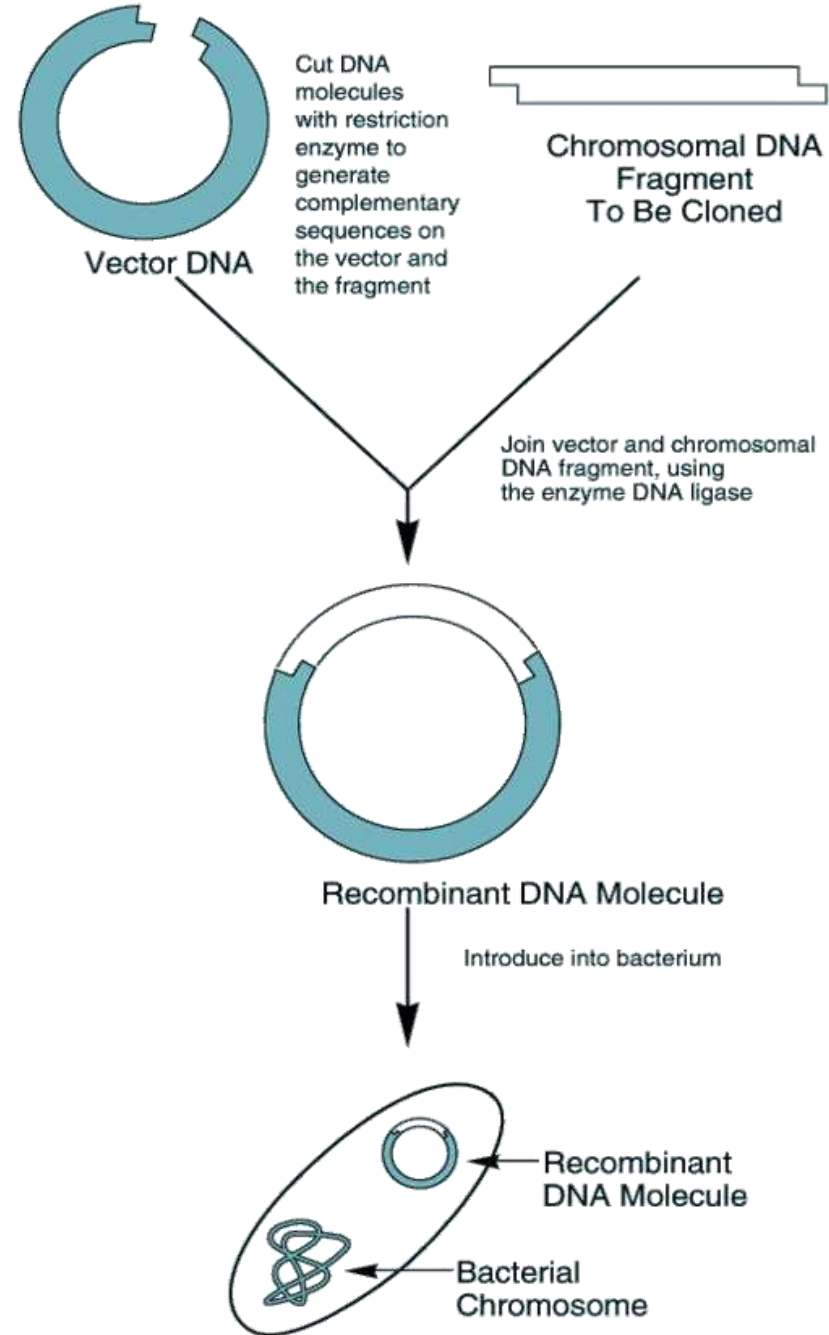


Production of Recombinant protein

- There are basically two methods for producing **recombinant proteins**.
- One is the molecular Cloning a laboratory method used to make **recombinant DNA**.
- The other method is the Polymerase chain reaction used to proceed the replication of any specific DNA sequence selected .
- The basic difference between the two methods is that molecular cloning incorporates the replication of the DNA within a living cell, whereas PCR replicates DNA in the test tube, without living cells.

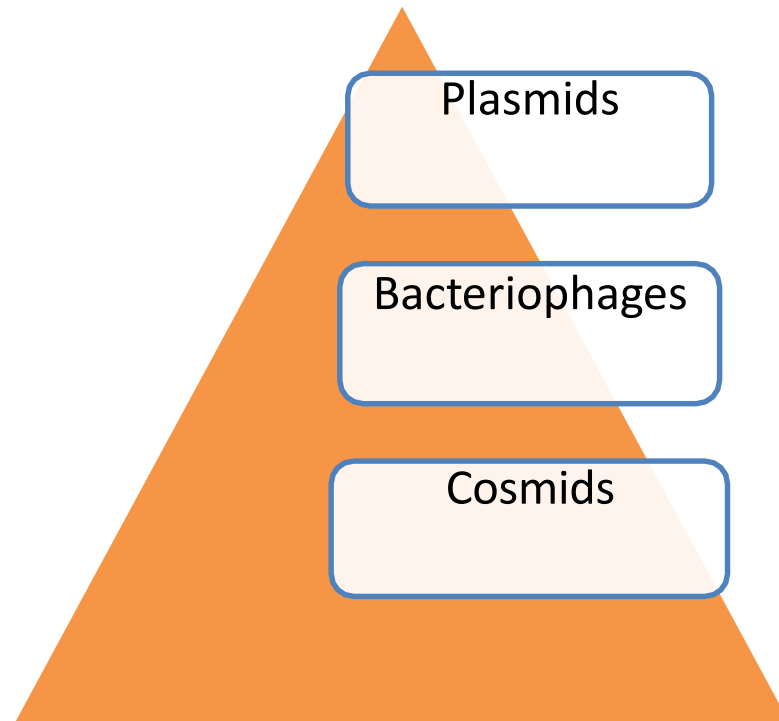
Cloning process

- Gene of interest is cut out with restriction enzymes (RE)
- Host plasmid (circular chromosome) is cut with same RE
- Gene is inserted into plasmid and ligated with ligase.
- New (engineered) plasmid inserted into bacterium (transform)



Vectors

- Self-replicating DNA molecules used to transfer foreign DNA segments between host cells.
- An ideal vector should be small in size, with single restriction endonuclease site.
- Three types of vectors



➤ **Plasmids**

- Bacteria contain extrachromosomal molecules of DNA called **plasmids** which are circular.
- pBR322 of E.coli is most popular and widely used plasmid vector.

➤ **Bacteriophages**

- Bacteriophages or simply phages are the viruses that replicate within the bacteria.
- Phages can accept foreign DNA fragments of 10-20 kb length.

➤ **Cosmids**

- These are specialized plasmids containing DNA sequence namely cos sites.
- Cosmids can carry larger fragments of foreign DNA compared to plasmids .i.e 20-50kb.

➤ Polymerize chain reaction



- A method for amplifying DNA segments using cycles of denaturation, annealing to primers, and DNA polymerase-directed DNA synthesis
- PCR copies a DNA molecule without restriction enzymes, vectors, or host cells .
- Faster and easier than conventional cloning.
- **First Step in PCR: Denaturation**
 1. DNA is heated to break the hydrogen bonds between the two polynucleotide strands.
- Two single-stranded DNA molecules serve as templates.

➤ **Second Step in PCR: Annealing**

2. Short nucleotide sequences (primers for DNA replication) are mixed with the DNA and bind to complementary regions on single-stranded DNA .
 - Takes place at lower temperature.
 - Primers are 20-30 nucleotides long, synthesized in the laboratory.

➤ **Third Step in PCR: DNA Synthesis**

3. The enzyme Taq polymerase is added to synthesize a complementary DNA strand.
 - Taq is a DNA polymerase from a bacterium found in hot springs.
 - These three steps make up one PCR cycle .



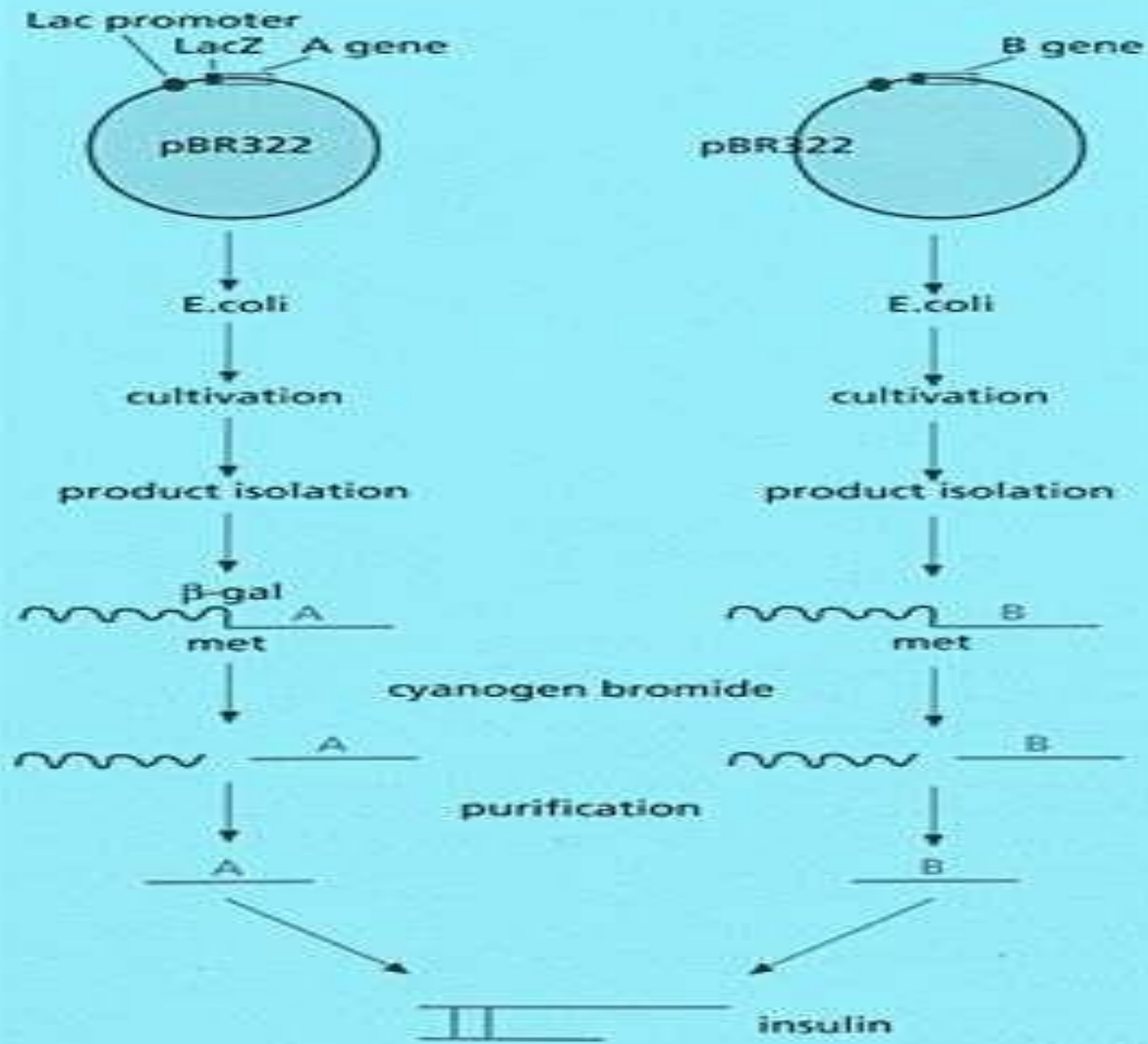
Production of recombinant Insulin

➤ Insulin

- Insulin is produced by β cells of islets of Langerhans of pancreas.
- Human insulin contains 51 amino acids ,arranged in two polypeptide chains.
- The chain A has 21 amino acids while chain B has 30 amino acids both are held together by disulfide bonds.



Insulin Production



APPLICATIONS

- Several proteins are created from recombinant DNA (recombinant proteins) and are used in medical applications.
- Hematopoietic growth factor.
- Interferon's
- Hormones
- Recombinant protein vaccines
- Tissue/bone growth factors and clotting factors
- Biological response modifiers
- Monoclonal/Diagnostic/Therapeutic antibodies
- Recombinant proteins is extensively used in biotechnology, medicine and research.

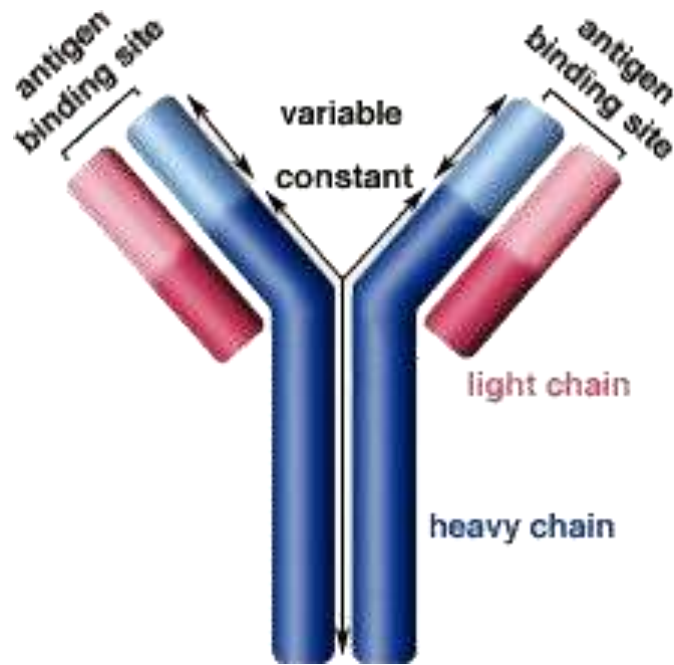


➤ Hematopoietic growth factor

- Product of blood cells in bone marrow of central axial skeleton is referred to as medullary hematopoiesis.
- While the mechanism of early stages of lineage commitment by bone marrow to particular type of blood cells remains elusive, the later stages of this process is driven by hematopoietic growth factor
- List of factors of recombinant origin

Product	Company	Indication
Thrombopoietin	Pharmacia	Thrombocytopenia
Erythropoietin	Amgen	Anaemia
Ancestim	Amgen	Blood cell transplantation

Antibody Structure

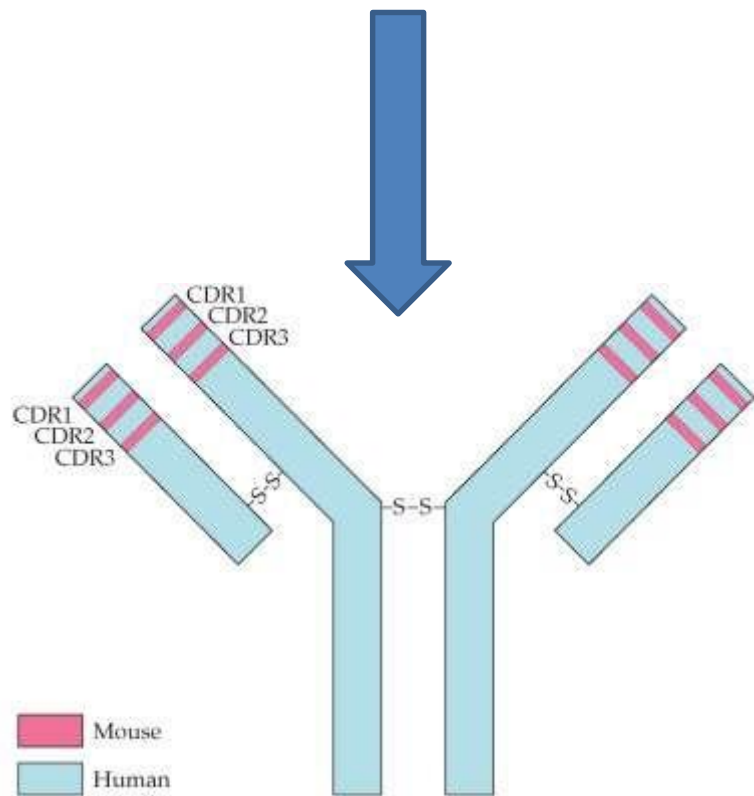


- Antibodies are immune system-related proteins called immunoglobulins. Each antibody consists of four polypeptides– two heavy chains and two light chains joined to form a "Y" shaped molecule.

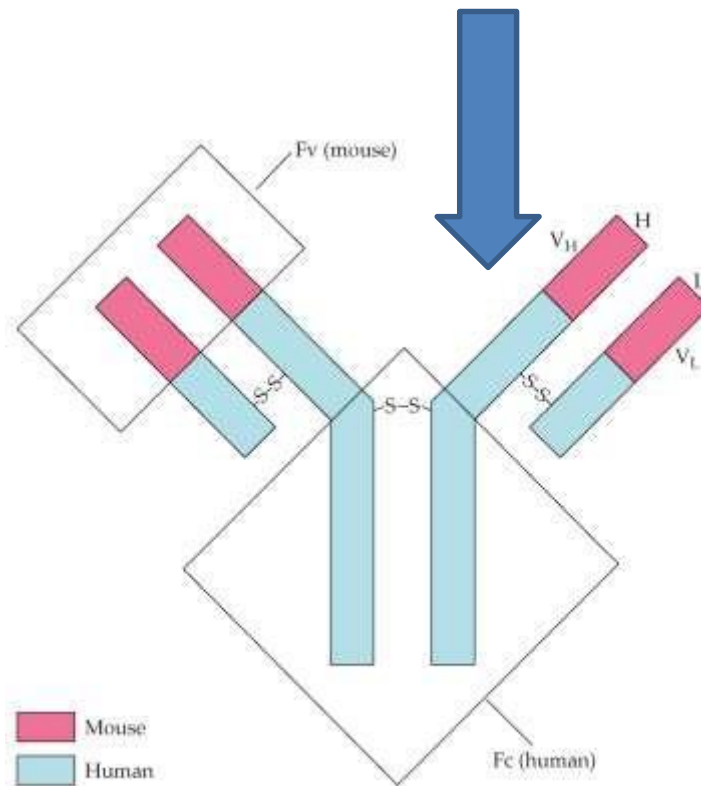
- The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110-130 amino acids, give the antibody its specificity for binding antigen. The variable region includes the ends of the light and heavy chains. Treating the antibody with a protease can cleave this region, producing Fab or fragment antigen binding that include the variable ends of an antibody.

- The constant region determines the mechanism used to destroy antigen. Antibodies are divided into five major classes, IgM, IgG, IgA, IgD, and IgE, based on their constant region structure and immune function.

Genetically engineered humanized ab



Genetically engineered chimeric ab

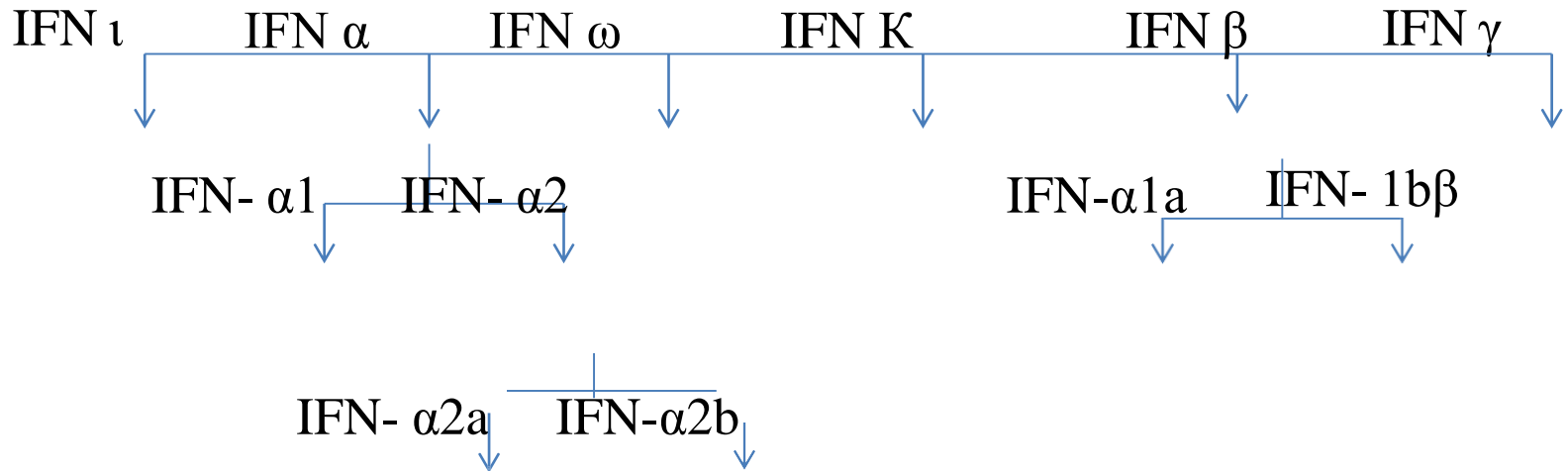


➤ Interferons

- In 1957 it was noted that infected by viruses produces protein called Interferon ,viral resistance to native cells.
- Inability to produce sufficient quality and inadequate purity, limits the clinical use of interferons.
- The problem of both purity and quality were resolved using recombinant DNA technology.



Types of recombinant IFN



➤ Hormones

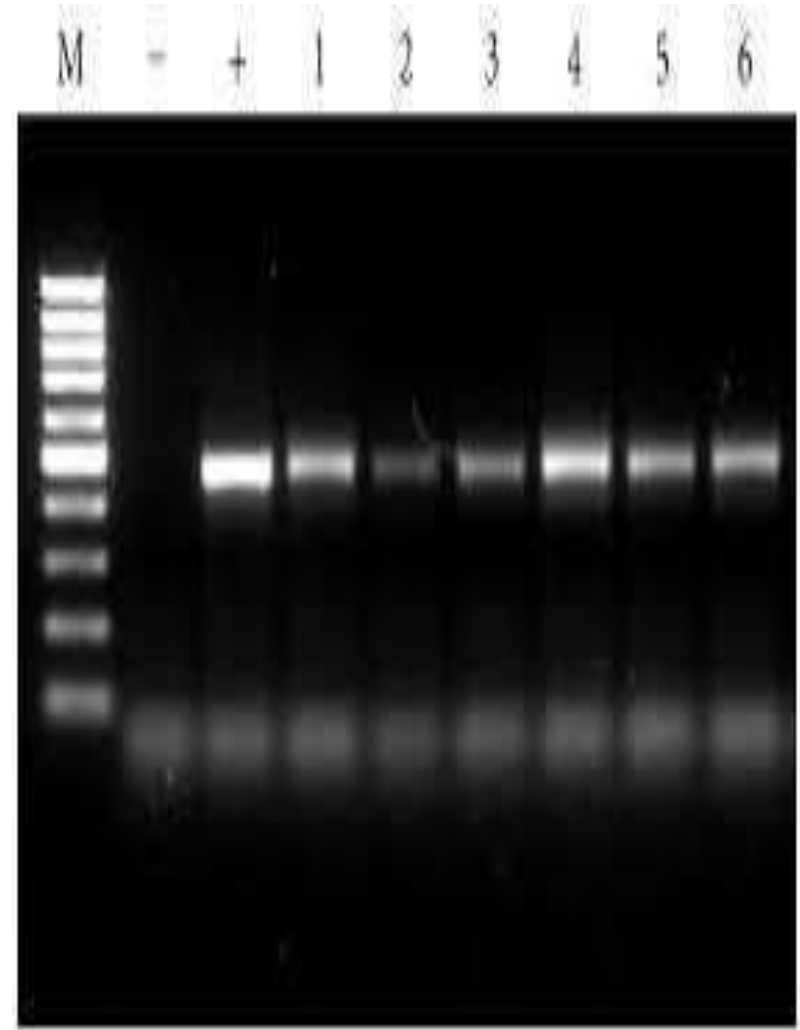
- Initially peptide and hormones used clinically were extracted and purified from animal or human source.
- Since these extraction may also contain animal proteins or peptides their use can lead to development of antibodies.
- There were limitations on use due to scarcity, contamination with other peptides and in some cases ineffectiveness.
- By introduction of recombinant DNA techniques, synthesizing of proteins and peptides takes place.
- In 1982 first recombinant protein hormone , insulin was introduced in USA.

➤ **List of hormones of recombinant origin**

Hormones	Company	Indication
Human chronic gonadotropin	Sereno	Breast cancer
Leptin	Amgen	Diabetes mellitus
Thyroid stimulating hormone	Genzyme	Recurrent thyroid cancer

Electrophoresis

- Most often used technique for protein products is sodium dodecyl sulphate polyacrylamide gel electrophoresis .
- Proteins are denatured by boiling in the SDS solution. All charges of protein are masked by negative charge of dodecyl sulphate.
- Thus protein moves on polyacrylamide gel strictly on basis of size of protein molecule.
- This technique is useful for determining molecular weight of proteins.
- For visualization of proteins on the gel reagents used are silver nitrate, coomassie brilliant blue dye.



List of products of recombinant origin

Product	Company	Indication
Alpha-glucosidase	Genzyme	Pompe's disease
Interleukin-4 receptor	Immunex	Asthma
Tumor necrosis factor receptor	Immunex	Rheumatoid arthritis
Vascular endothelial growth factor	Genvec	Cardiovascular disorders
HIV vaccine	Chiron	AIDS
Prostvac	Therion	Prostate cancer
Neurex	Xoma	Cystic fibrosis

CONCLUSION

- Recombinant technology is mostly used in production of insulin, human growth hormone, vaccines ,Interferons etc.
- Recombinant proteins are used in medical applications, particularly as medications and vaccines.
- Development of improved drug delivery system.
- Recombinant technology is that it allows introduction of modifications into proteins at desired positions.



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❖ WEBSITES

- ✓ <http://www.innovateus.net/science/what-are-recombinant-proteins> .
- ✓ http://en.wikipedia.org/wiki/Recombinant_DNA .
- ✓ <http://www.accessexcellence.org/RC/VL/GG/plasmid.php>