Techniques used to produce Biopharmaceuticals

Lecture Two

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Dr. Ahmed Najim Abood
Assistant Professor in Pharmaceutics
ahmed.abood@uobasrah.edu.iq

Techniques used to produce Biopharmaceuticals

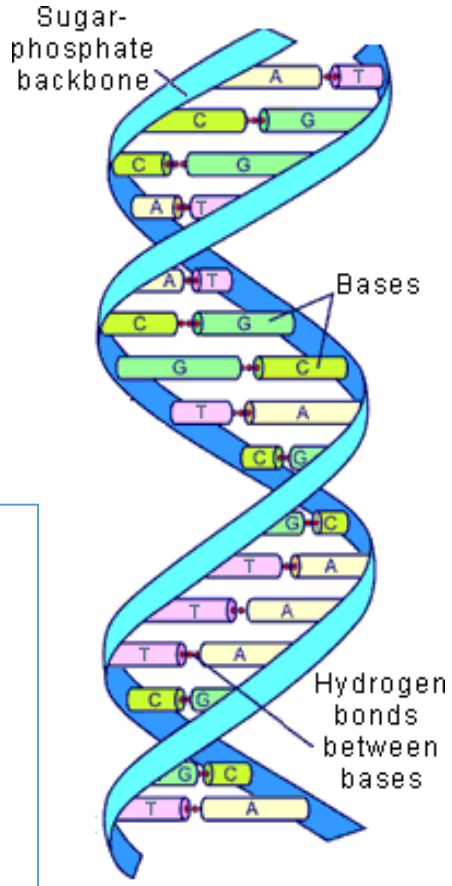
There are several techniques:
Such as

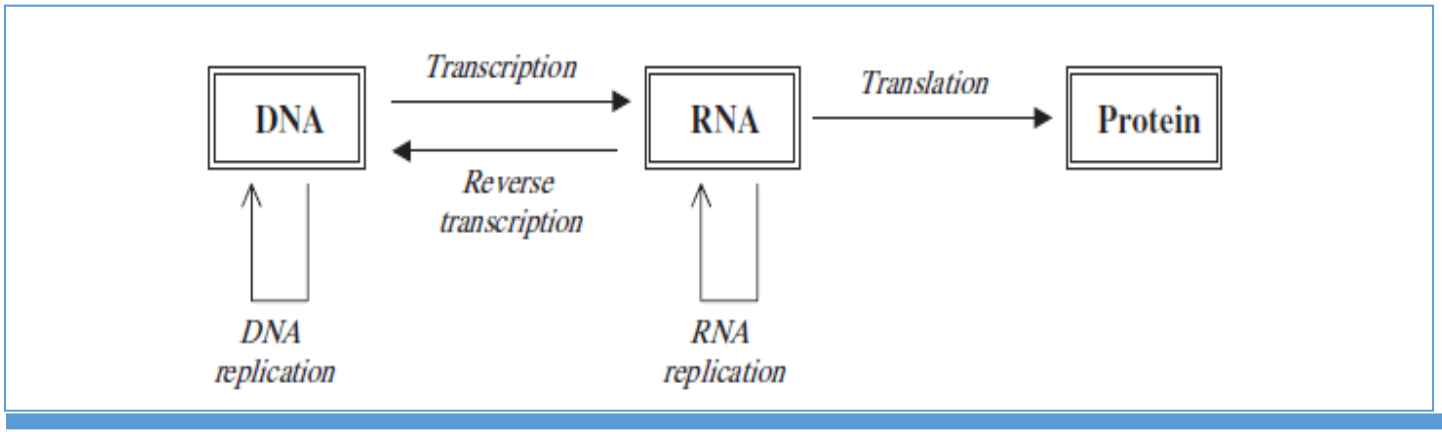
1) **rDNA technology**
2) **mAb technology**
3) **Gene therapy**

Recombinant DNA Technology

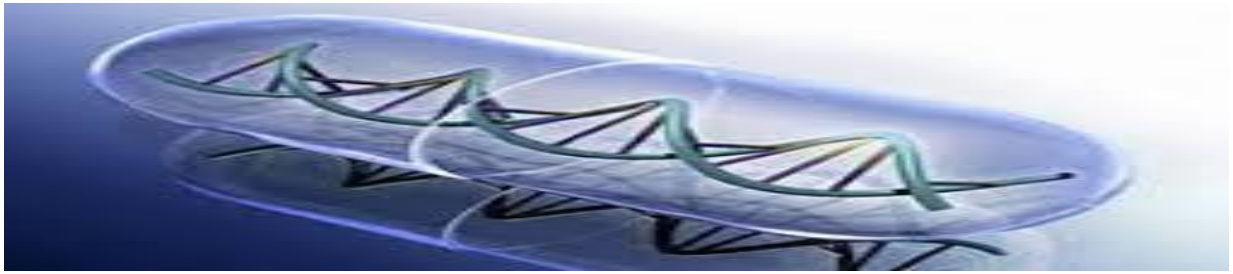
It is the predominant technology in biotechnology from the 1980s to the present, which has been used to create over 40% of the commercially available biotech products.

- DNA, deoxyribonucleic acid, has been called the substance of life.
- DNA constitutes genes, allowing cell to reproduce and maintain life.
- It also plays an essential role in the production of proteins for cellular maintenance and function.





- This technology includes identification, isolation and insertion of naturally occurring or synthetic nucleotide sequence (gene of interest) into a vector (plasmid or bacteriophage) to form a recombinant DNA molecule and production of large quantities of that gene fragment or product encoded by that gene.



- The recombinant plasmid is introduced into a suitable host organism (transformation process) to ensure the efficient expression of the desired gene product.

What are the restrictions of this technology?

- It is a quite multifaceted and complex with much potential for contamination, variation, and alteration, leading to poor outcomes, e.g., a deteriorated product, a degraded protein, viral or bacterial contamination, poor yield, an immune reaction in patients, and even different protein.

What are the steps of rDNA technology?

1) Finding **a protein** responsible for some biological effect in the human body.

2) Isolation of the human gene (DNA) responsible for the desired protein and may obtained by organic DNA synthesis.

3) Cloning of the gene and expression of the protein by the gene.

- **Cloning** is the reproduction of the target human gene in a non-human cell.
- **Expression** is the production of the target human protein by a non-human cell containing the human gene.

- These processes require a vector (a plasmid or bacteriophage) for the DNA (genes), so that the gene can be carried into a host cell.
- A bacterial plasmid is a circular piece of DNA that is transferable between cells (therefore, a carrier), will accept the insertion of a human gene, and will allow the human gene to be turned on.

- Plasmids are further manipulated to maximize their function with the addition of promoter, enhancer, and operator DNA sequences.
- The plasmid must be cut open to accept the human DNA (gene) by unique enzymes **(bacterial restriction endonucleases)**, each of which is highly specific to a certain nucleic acid sequence that can match a terminal end of the human gene.

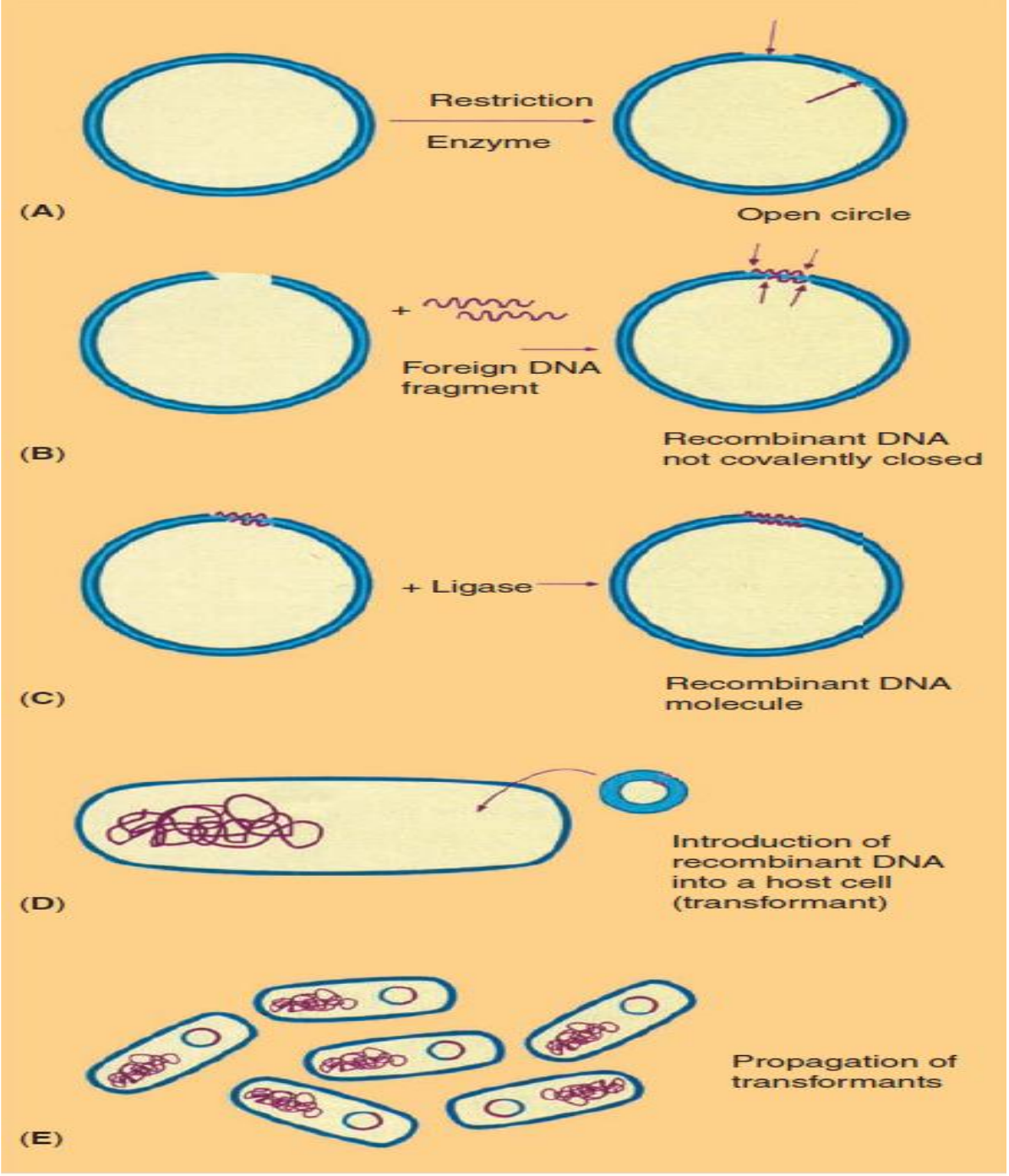


Figure 10 Principle of cloning a foreign DNA fragment.

- These ‘‘sticky’’ ends of the opened bacterial plasmid and the human gene permit recombination of the DNA, under the influence of **a ligase enzyme**, resulting in an r-DNA molecule containing a human gene inserted into a bacterial plasmid.

- Next, the r-DNA molecule is inserted into a host cell ??, which serves to produce all of its routine proteins, and the cell manufactures the human protein from the human gene that it carries.

- rDNA transfer may be occurred by bacteriophage

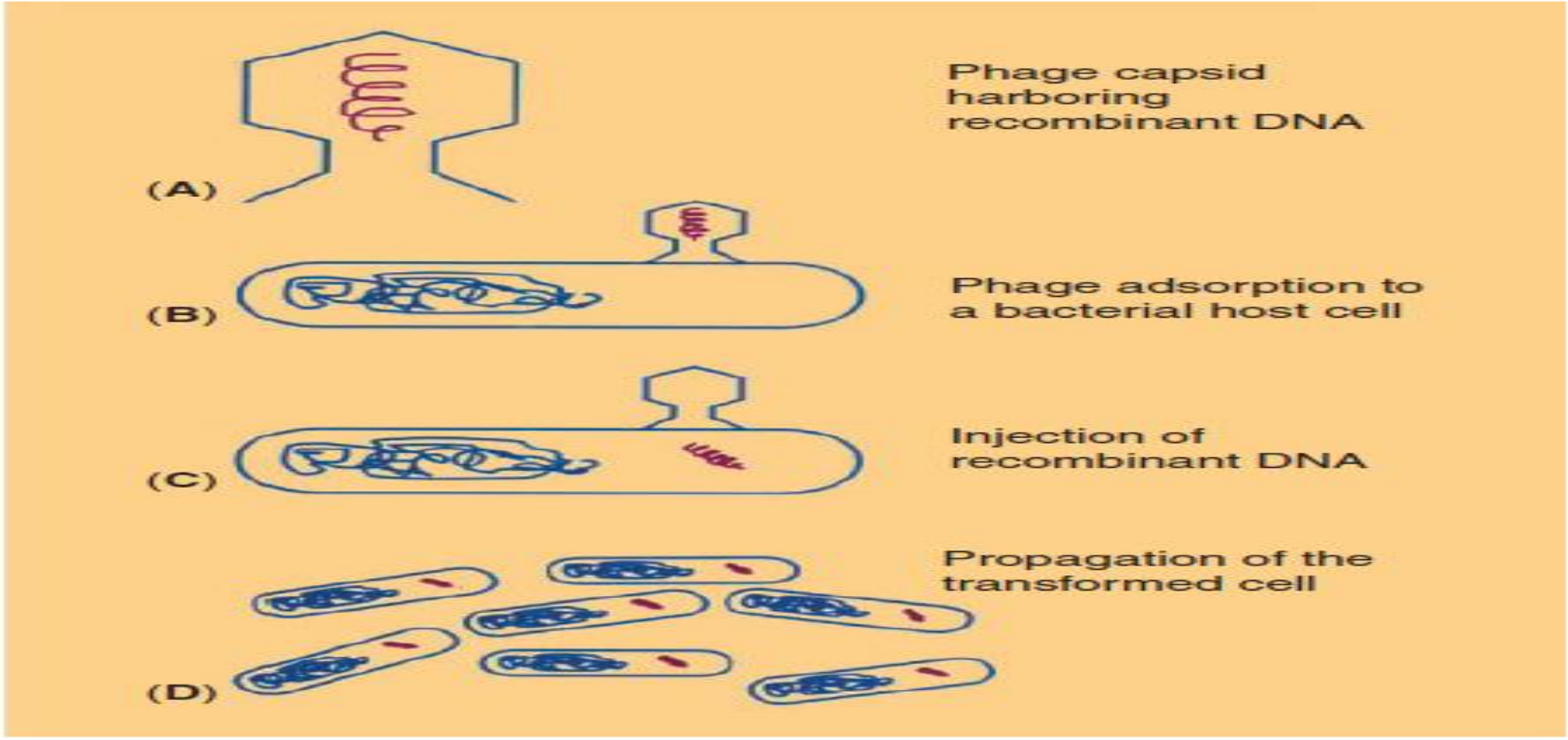


Figure 11 Phage as a mediator for transfer of recombinant DNA.

- The host cells can be bacteria, usually E. coli, yeast cells, and mammalian cells, usually Chinese hamster ovary cells or baby kidney hamster cells.
- This unique newly created cell and its offspring, created in the laboratory, are called **the master working cell bank**.

- **The fermentation** or **(cell culture phase)** in presence of appropriate fortified growth media.
- The bacterial host cells will proceed to produce proteins, intracellularly with feeding and removing waste from the media for more productivity followed by harvesting the cell to obtain the pure bulk protein by **purification**.

- The cells are removed from the liquid in the fermenters by **centrifugation** into a cell paste, which is centrifuged again to break out proteins from the cells.
- The protein mixture is then run through an extraction process, often **(HPLC)** to separate the target protein from all other proteins.

- The final phase is **formulation** using the best mix of fluids, buffers, stabilizers, and minerals to achieve optimal protein stability, maximal shelf life, and patient acceptability. (Sterile water, normal saline, and dextrose 5% in water are three common diluents).

What are the properties of ideal host cell?

1) A short reproductive life cycle and long-term viability in an in vitro setting.

2) The ability to accept bacterial plasmids.

3) Substantial productive capacity (yield) for proteins.

4) The ability to produce the human protein consistently without its alteration (possibly glycosylation of the protein).

5) Ease of manufacturing in the later scale-up process, as low as possible cost in manufacturing, and patentability to protect the intellectual property.