PHARMACEUTICAL APPLICATION OF RECOMBINANT DNA TECHNOLOGY

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INTRODUCTION

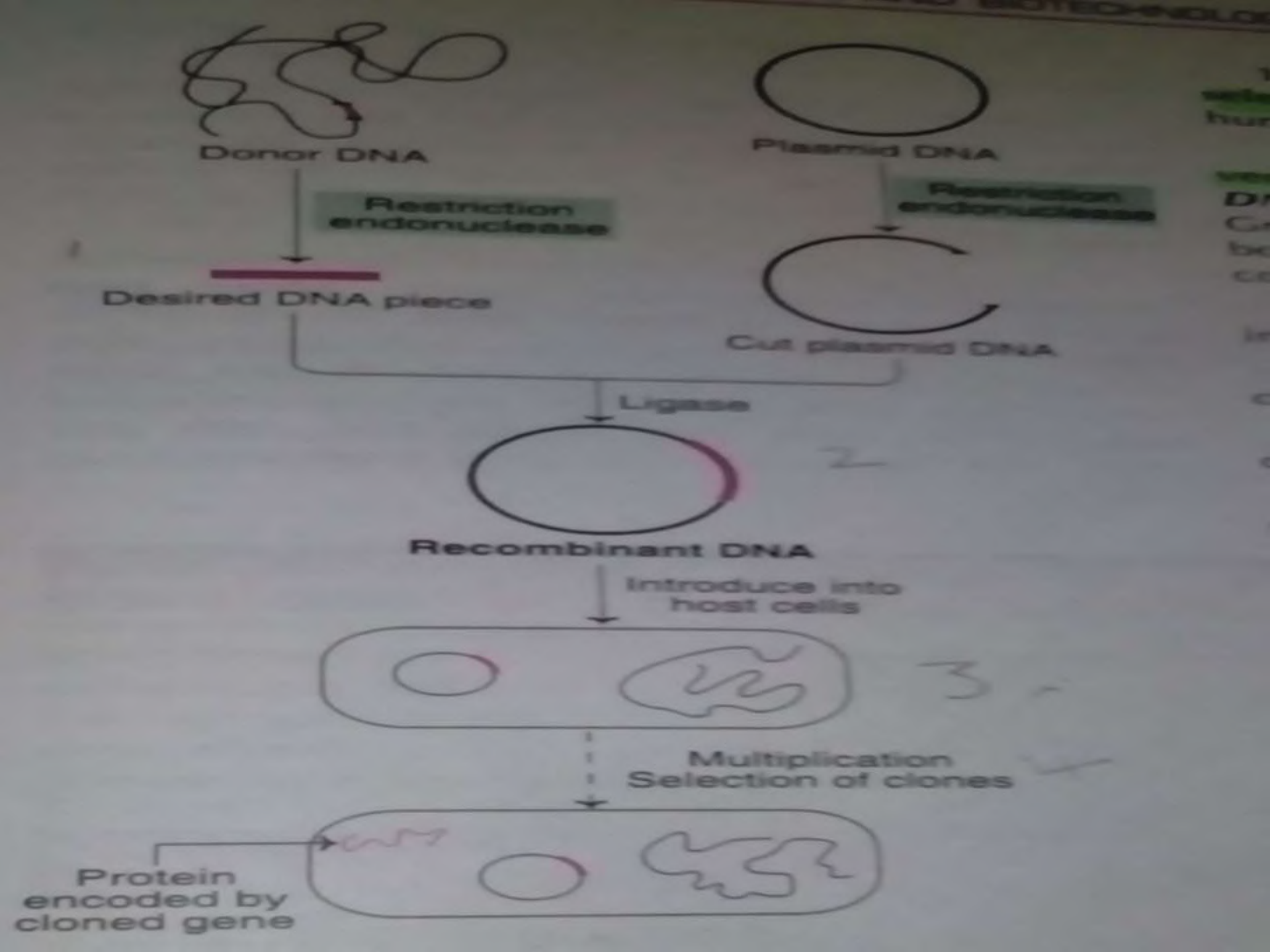
- Recombinant DNA molecules are sometimes called **chimeric DNA**, because they are usually made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

HISTORY OF RECOMBINANT DNA TECHNOLOGY

- Recombinant DNA technology in the experiments performed by Boyer and Cohen in 1973.

- In this, they recombined two plasmids & cloned the new plasmid in E. coli.

- In later experiments the genes of a frog successfully transplanted, and expressed in E.coli.


Donor DNA
Restriction endonuclease
Desired DNA piece
Plasmid DNA
Restriction endonuclease
Cut plasmid DNA
Ligase
Recombinant DNA
Introduce into host cells
Multiplication
Selection of clones
Protein encoded by cloned gene

BASIC PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

- Selection of the desired piece of DNA
- Insertion of selected DNA into a cloning vector to create a RECOMBINANT DNA or CHIMERIC DNA
- Introduction of recombinant vector into host cell (E.g. Bacteria)
- Multiplication & selection of clones containing the recombinant molecules
- Expression of the gene to produce the desired product

Applications of recombinant DNA technology

Recombinant chymosin

- found in rennet, is an enzyme required to manufacture cheese. It was the first genetically engineered food additive used commercially. Traditionally, processors obtained chymosin from rennet, a preparation derived from the fourth stomach of milk-fed calves. Scientists engineered a non-pathogenic strain of *E. coli* bacteria for large-scale laboratory production of the enzyme. This microbiologically produced recombinant enzyme, identical structurally to the calf derived enzyme.

Recombinant blood clotting factor VIII

- *a blood-clotting protein that is administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation. Before the development of recombinant factor VIII, the protein was obtained by processing large quantities of human blood from multiple donors, which carried a very high risk of transmission of blood borne infectious diseases, for example HIV and hepatitis B.*

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 (RT-PCR). Development of the RT-PCR test was made possible by the molecular cloning and sequence analysis of HIV genomes. HIV testing page from US Centers for Disease Control (CDC)

DAIGNOSIS OF INFECTIOUS DISEASES

Malaria:

A specific DNA diagnostic test for identification of the current infection of P. falciparum has been developed. This is carried by using a DNA probe that can bind and hybridize with a DNA fragment of P. falciparum genome .

Production of recombinant insulin:.

•Inserting human insulin gene and the promoter gene of lac operon on to the plasmids of *E.coli.*

•Recently the procedure involves insertion of genes for insulin A chain and B chain separately to the plasmids of different*E.coli*cultures.

•The lac operon system is used for expression of both genes. the presence of lactose in the culture medium induces the synthesis of insulin A and B chains in separate cultures.

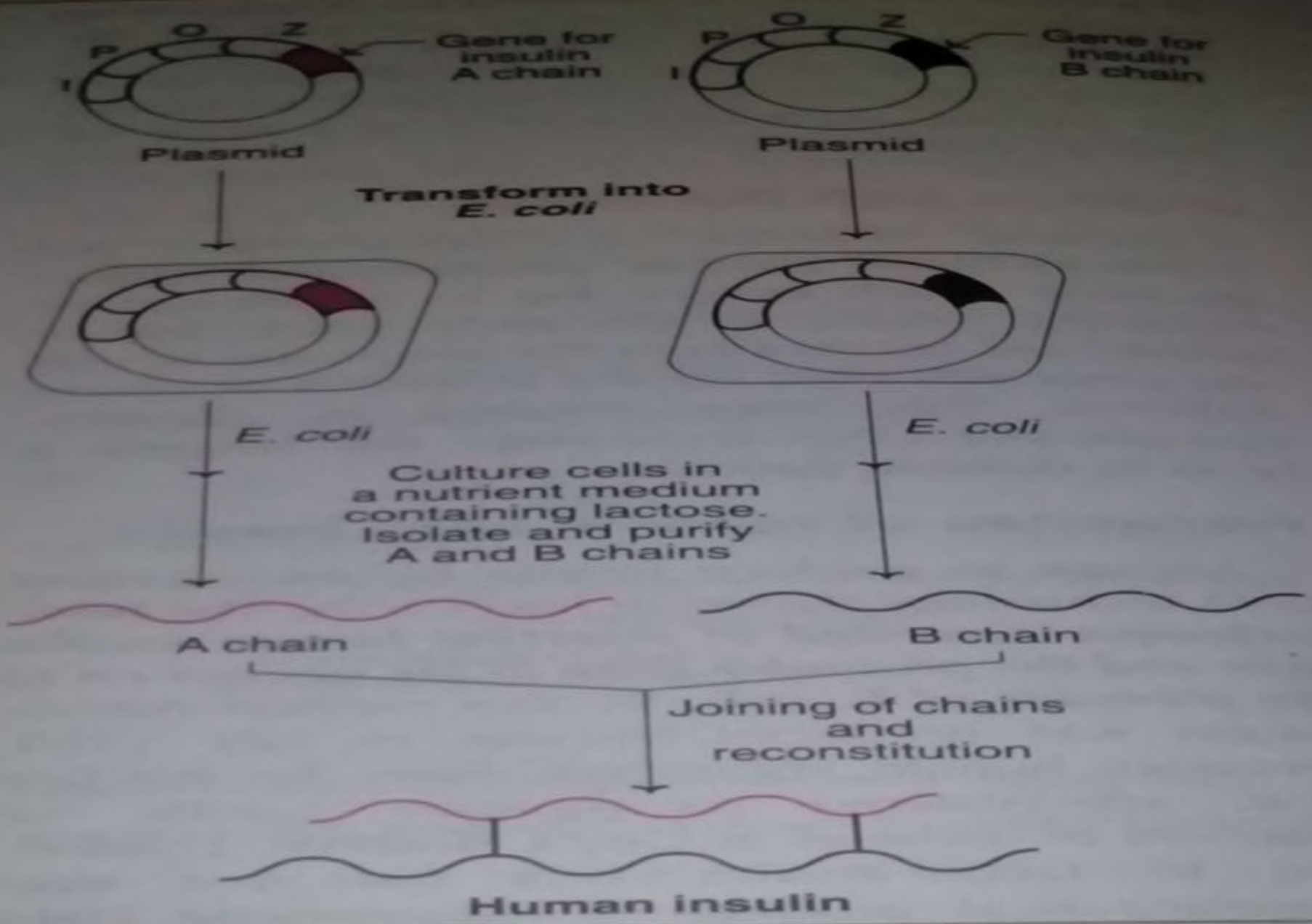


***Fig. 27.28** : The production of recombinant insulin in E. coli (I-Inducer gene, P-Promoter gene,*

Production of recombinant HGH

•Biotechnologists can now produce HGH by genetic engineering.

•The technique adopted is quite comparable with that of insulin production.

•The procedure essentially consists of inserting HGH gene into *E.coli* plasmid, culturing the cells and isolation of the HGH from the extracellular medium.

Recombinant 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.

Steps involved:

Production of recombinant vaccines involves the following steps:

(i) Protein which is crucial to the growth and development of the causative organism be identified.

(ii) The corresponding gene is then isolated applying various techniques.

(iii) This gene is then integrated into a suitable expression vector

(iv) This rDNA is used as vaccines or is introduce into another host organism

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Thank You