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Application of rDNA Technology and Genetic Engineering in Pharmaceutical Production



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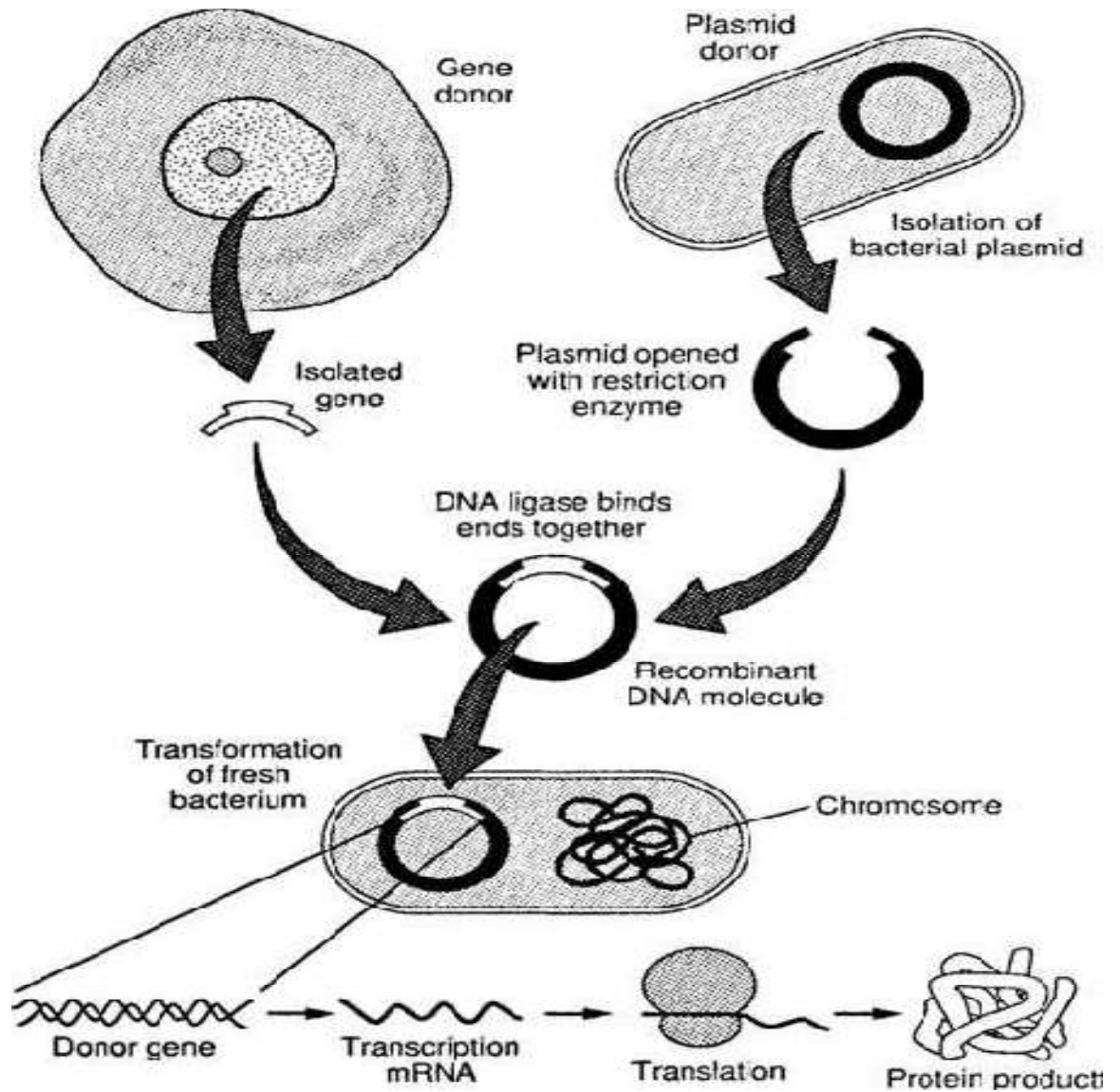
Recombinant DNA Technology

- Manipulation of DNA sequences and the construction of chimeric molecules, provides a means of studying how a specific segment of DNA works
- Studies in bacteria and bacterial viruses have led to methods to manipulate and recombine DNA
- Once properly identified, the recombinant DNA (rDNA) molecules can be used in various ways useful in medicine and human biology



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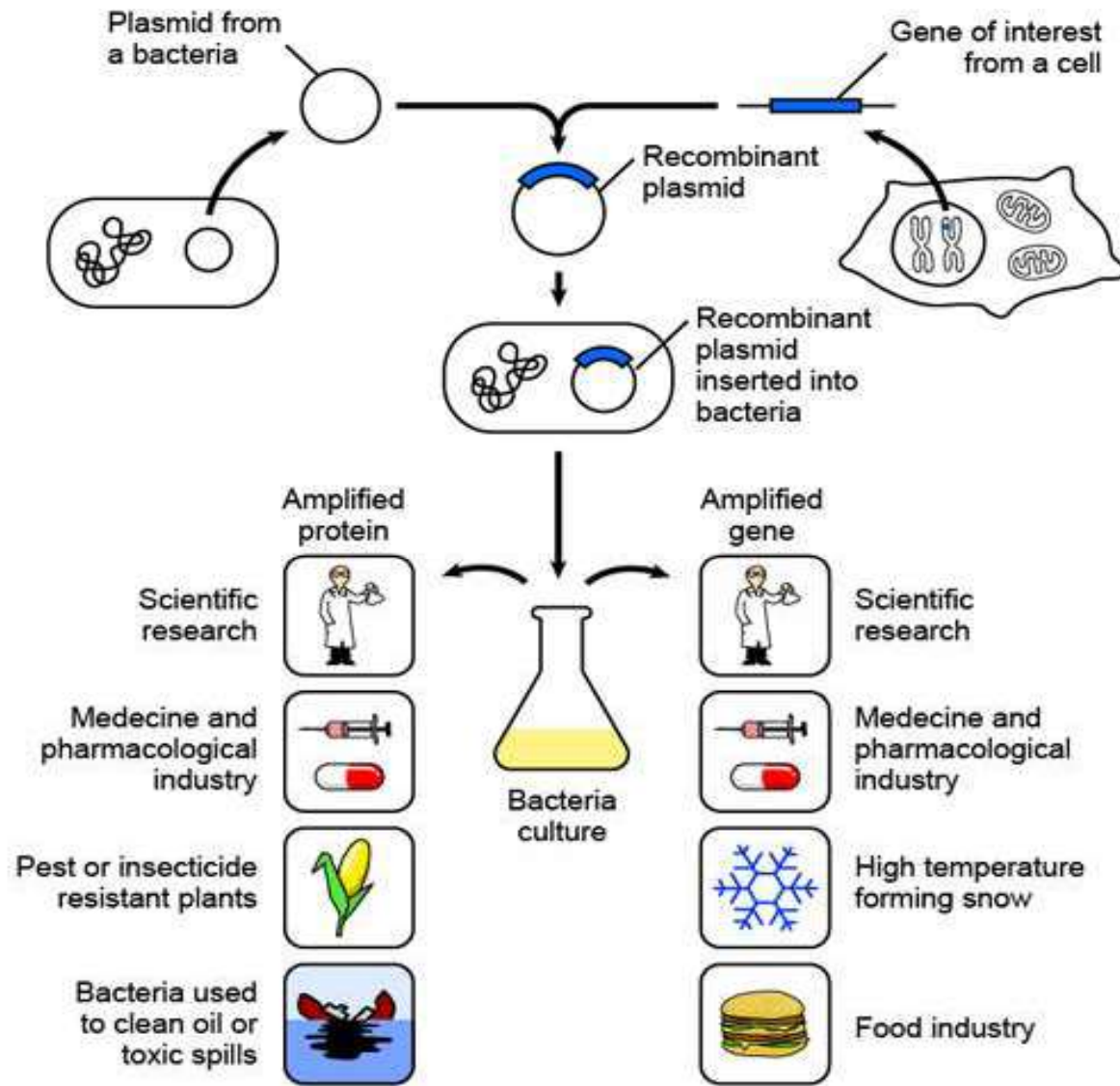
Procedure





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Recombinant Pharmaceuticals

- A number of human disorders can be traced to the absence or malfunction of a protein normally synthesized in the body.
- Most of these disorders can be treated by supplying the patient with the correct version of the protein
- Hence, modern pharmaceutical manufacturing frequently relies upon recombinant drugs.



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Recombinant Pharmaceuticals

1. Human Insulin

- Human Growth Hormone
- Human blood clotting factors

2. Vaccines (Hepatitis B)

- Monoclonal Antibodies

3. Interferons

- Antibiotics & other secondary metabolites



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1. HUMAN INSULIN

- Earliest use of recombinant technology
- Modify *E.coli* cells to produce insulin; performed by Genentech in 1978
- Bovine and porcine insulin used but induced immunogenic reactions
- Also, there were many purification and contamination hassles.
- To overcome these problems, researchers inserted human insulin genes into a suitable vector (*E.coli*)



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Producing Recombinant Insulin

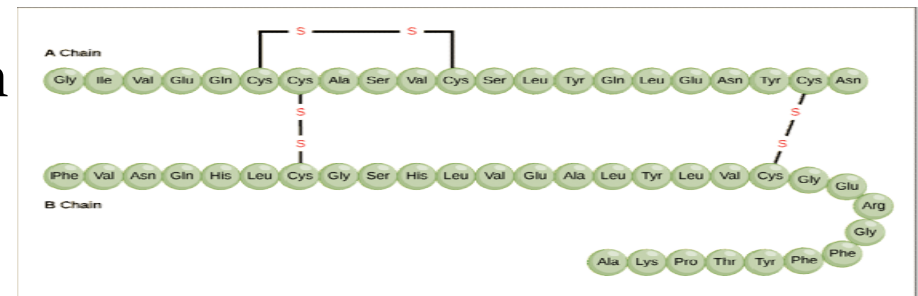
- First, scientists synthesized genes for the two insulin A & B chains.
- They were then inserted into plasmids along with a strong lacZ promoter.
- The genes were inserted in such a way that the insulin & β -galactosidase residues would be separated by a methionine residue. This is so that the insulin A & B chains can be separated easily by adding cyanogen bromide.



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Producing Recombinant Insulin

- The vector was then transformed into *E.coli* cells.
- Once inside the bacteria, the genes were "switched-on" by the bacteria to translate the code into either the "A" chain or the "B" chain proteins found in insulin

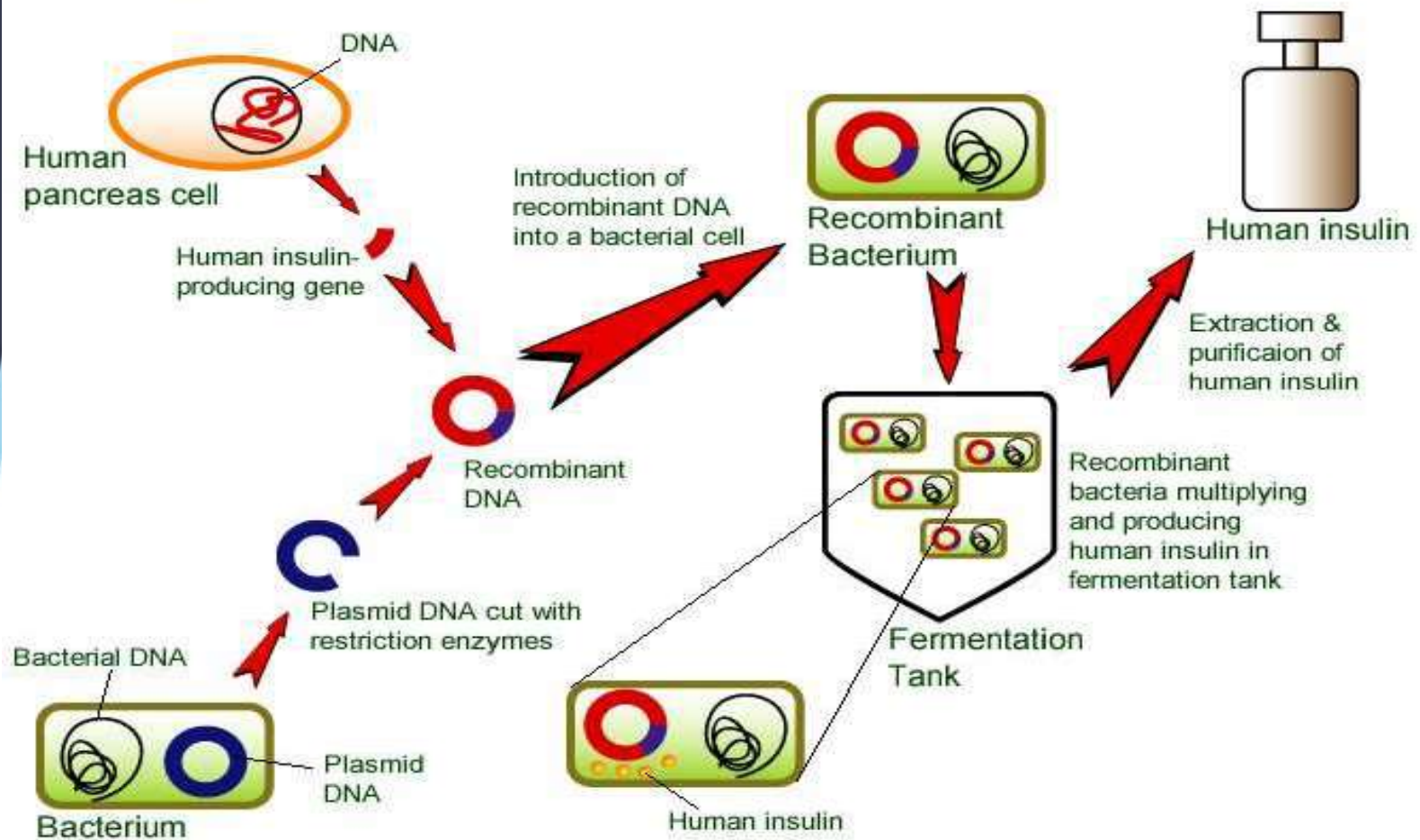


- The purified insulin A and B chains were then attached to each other by disulphide bond formation under laboratory conditions



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Human Insulin Production





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2. RECOMBINANT VACCINES

Two types:

(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.



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Recombinant protein vaccines:

- A pathogen produces its proteins in the body which elicit an immune response from the infected body.
- The gene encoding such a protein is isolated from the causative organism
- This DNA is expressed in another host organism, like genetically engineered microbes; animal cells; plant cells; insect larvae etc, resulting in the release of appropriate proteins.
- These when injected into the body, causes immunogenic response against the corresponding disease providing immunity.



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DNA vaccines:

- Refers to the recombinant vaccines in which the DNA is used as a vaccine.
- The gene responsible for the immunogenic protein is identified, isolated and cloned with corresponding expression vector.
- Upon introduction into the individuals to be immunized, it produces a recombinant DNA.
- This DNA when expressed triggers an immune response and the person becomes successfully vaccinated.
- **The mode of delivery of DNA vaccines include:** direct injection into muscle; use of vectors like adenovirus, retrovirus etc; *in-vitro* transfer of the gene into autologous cells and re-implantation of the same and particle gun delivery of the DNA.



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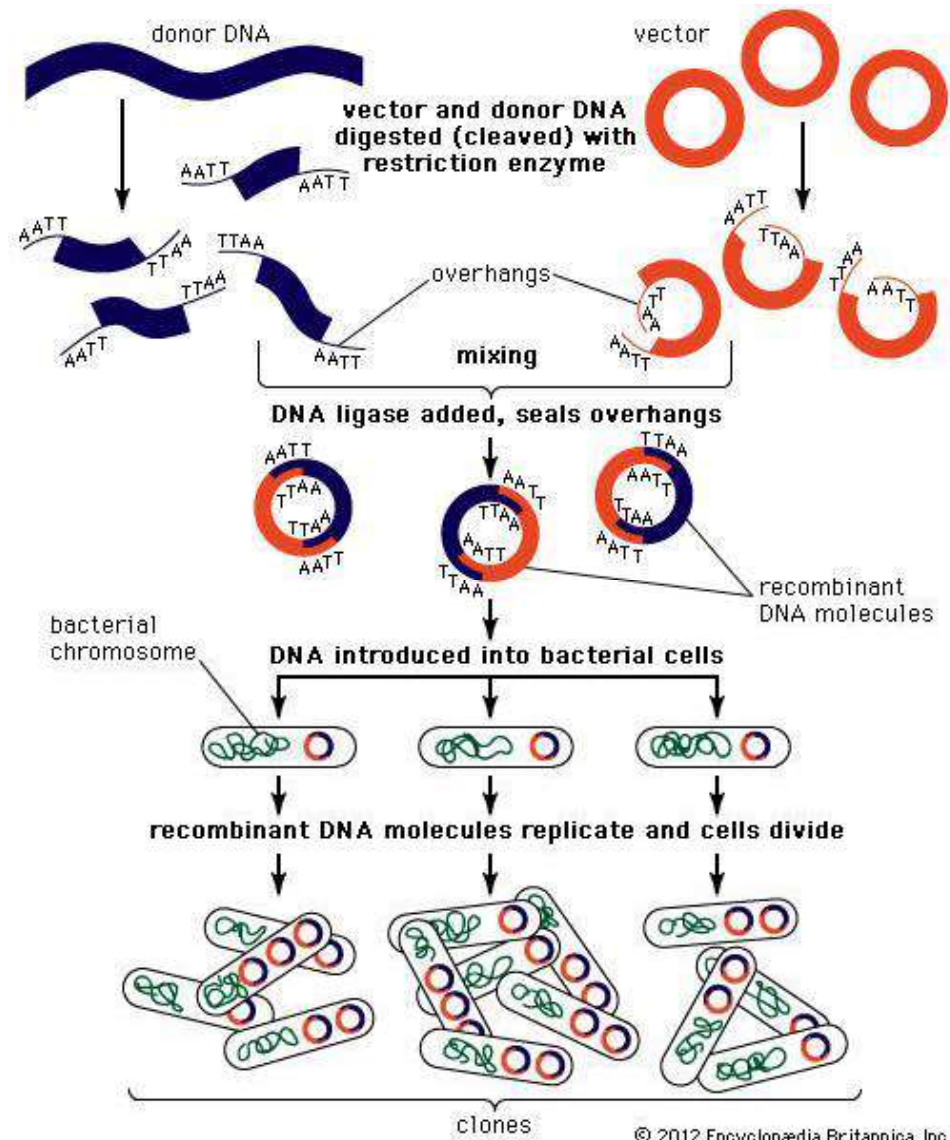
DNA vaccines:

- In certain cases, the responsible gene is integrated into live vectors which are introduced into individuals as vaccines.
- This is known as *live recombinant vaccines*. Eg: vaccinia virus. Live vaccinia virus vaccine (VV vaccine) with genes corresponding to several diseases, when introduced into the body elicit an immune response but does not actually cause the diseases.



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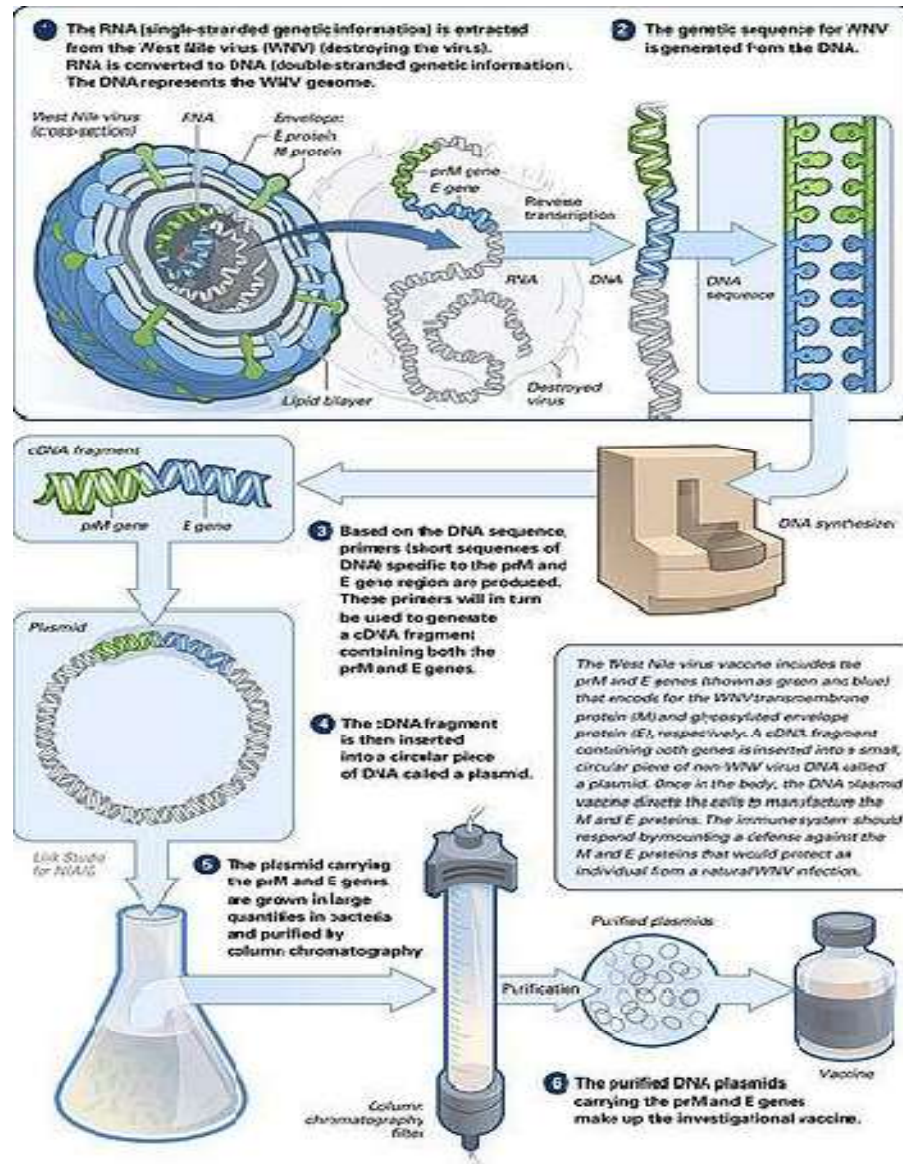
Process





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Process





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3. INTERFERONS

- Interferons (IFNs) are a group of signaling proteins made and released by host cells in response to the presence of pathogens, such as viruses, bacteria, parasites or tumour cells.
- In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses.



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Production of Recombinant Interferons

- Recombinant DNA technology has proved the most satisfactory route to the large scale production of human interferons.
- The genes of all three types of Human IFN have been cloned in micro-organisms and expression obtained.
- Human IFN- β and γ produced in this manner lack the glycosylation present in the naturally occurring substances but this does not affect their specific activity.



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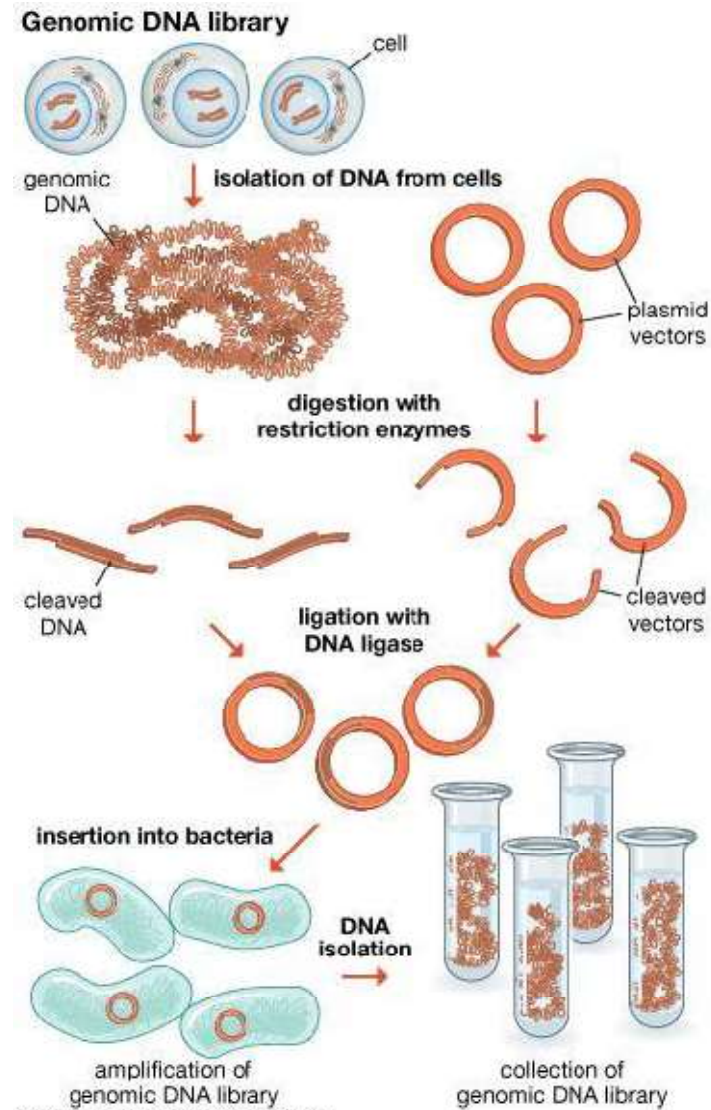
Production of Recombinant Interferons

- Greatly improved methods of purification, including **immuno-adsorption chromatography** on monoclonal antibody columns, are now available so there should be no difficulty in supplying adequate amounts of very pure interferon of all three types although, up till now, only Human IFN- α has been readily available.



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Process



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THANK YOU

HAPPY TO ANSWER IF YOU HAVE ANY QUERIES.....?