Application of rDNA Technology and Genetic Engineering in Pharmaceutical Production

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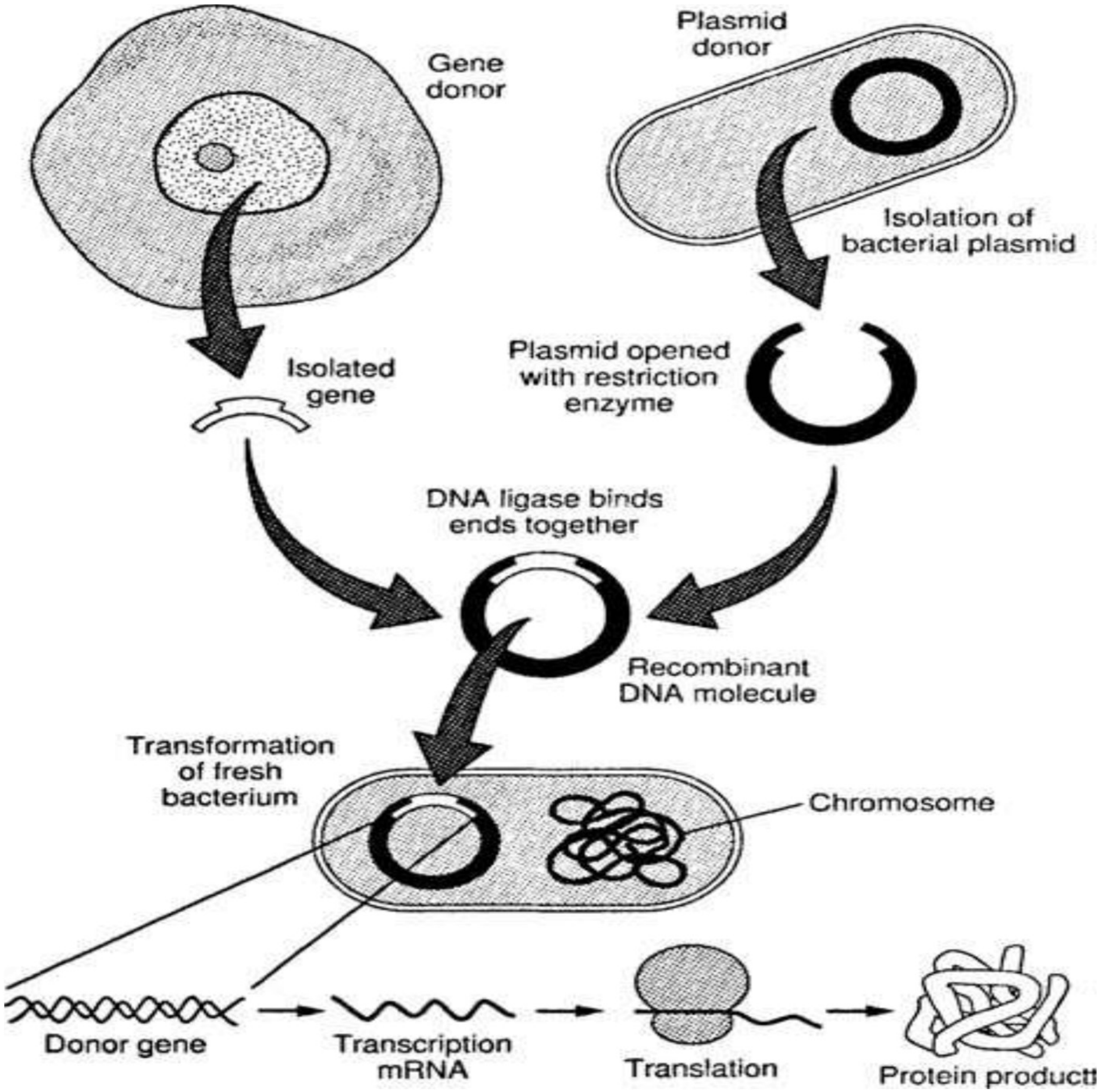
Recombinant DNA Technology

- Manipulation of DNA sequences and the construction of chimeric molecules, provides a means of studying how a specific segment of DNA works

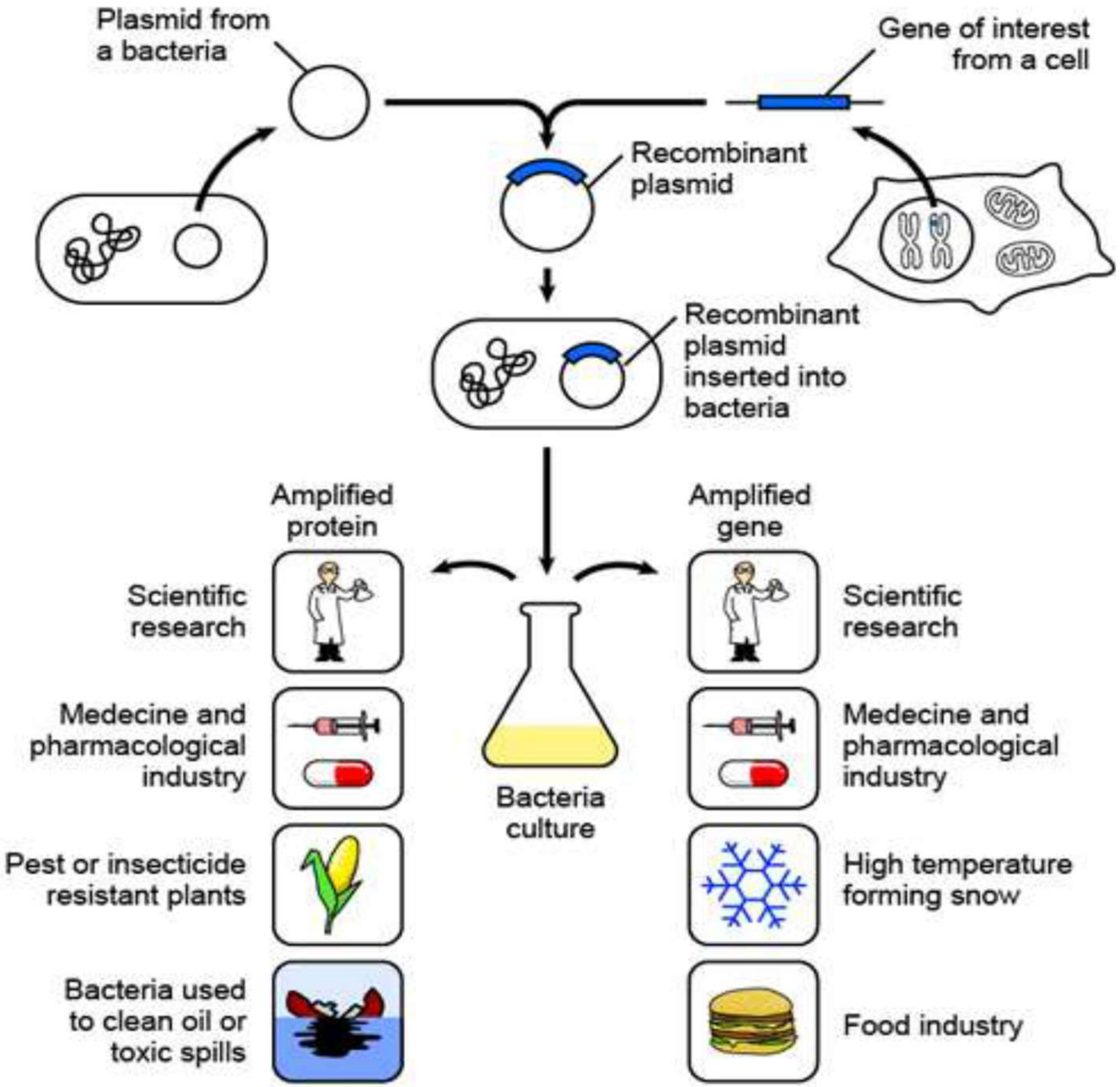
- Studies in bacteria and bacterial viruses have led to methods to manipulate and recombine DNA

- Once properly identified, the recombinant DNA (rDNA) molecules can be used in various ways useful in medicine and human biology

Procedure



Gene donor
Plasmid donor
Isolation of bacterial plasmid
Isolated gene
Plasmid opened with restriction enzyme
DNA ligase binds ends together
Recombinant DNA molecule
Transformation of fresh bacterium
Chromosome
Donor gene
Transcription mRNA
Translation
Protein productt



Plasmid from a bacteria
Gene of interest from a cell
Recombinant plasmid
Recombinant plasmid inserted into bacteria
Amplified protein
Amplified gene
Scientific research
Medecine and pharmacological industry
Pest or insecticide resistant plants
Bacteria used to clean oil or toxic spills
Bacteria culture
Scientific research
Medecine and pharmacological industry
High temperature forming snow
Food industry

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.

Recombinant Pharmaceuticals

- Human Insulin
- Human Growth Hormone
- Human blood clotting factors
- Vaccines (Hepatitis B)
- Monoclonal Antibodies
- Interferons
- Antibiotics & other secondary metabolites

HUMAN INSULIN

- Earliest use of recombinant technology
- Modify *E.coli* cells to produce insulin; performed by Genentech in 1978
- Prior, 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*)

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.

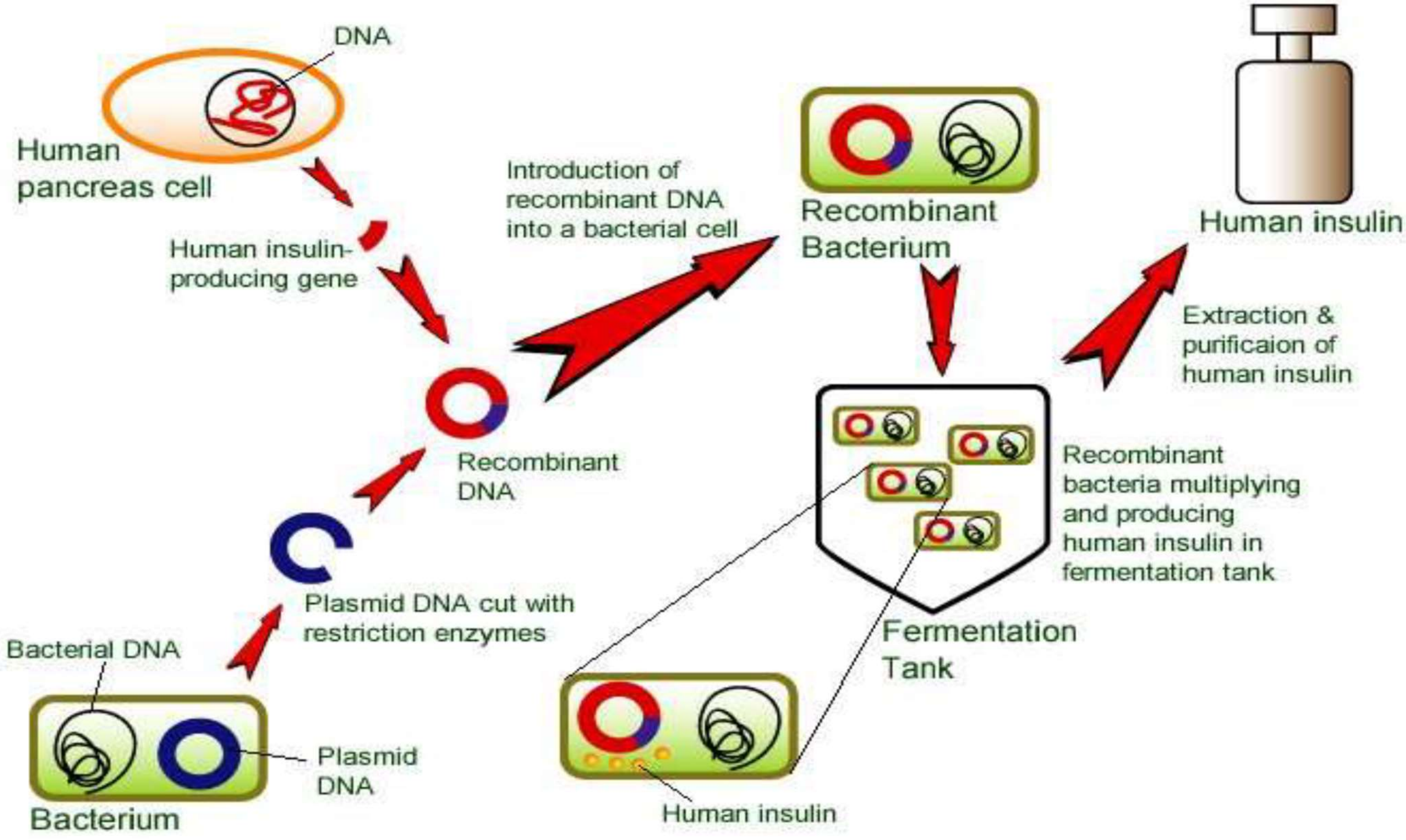
Producing Recombinant Insulin

- 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

Human Insulin Production



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.

Production of Recombinant Interferons

- Recombinant DNA technology has proved the most satisfactory route to the large scale production of human interferons.

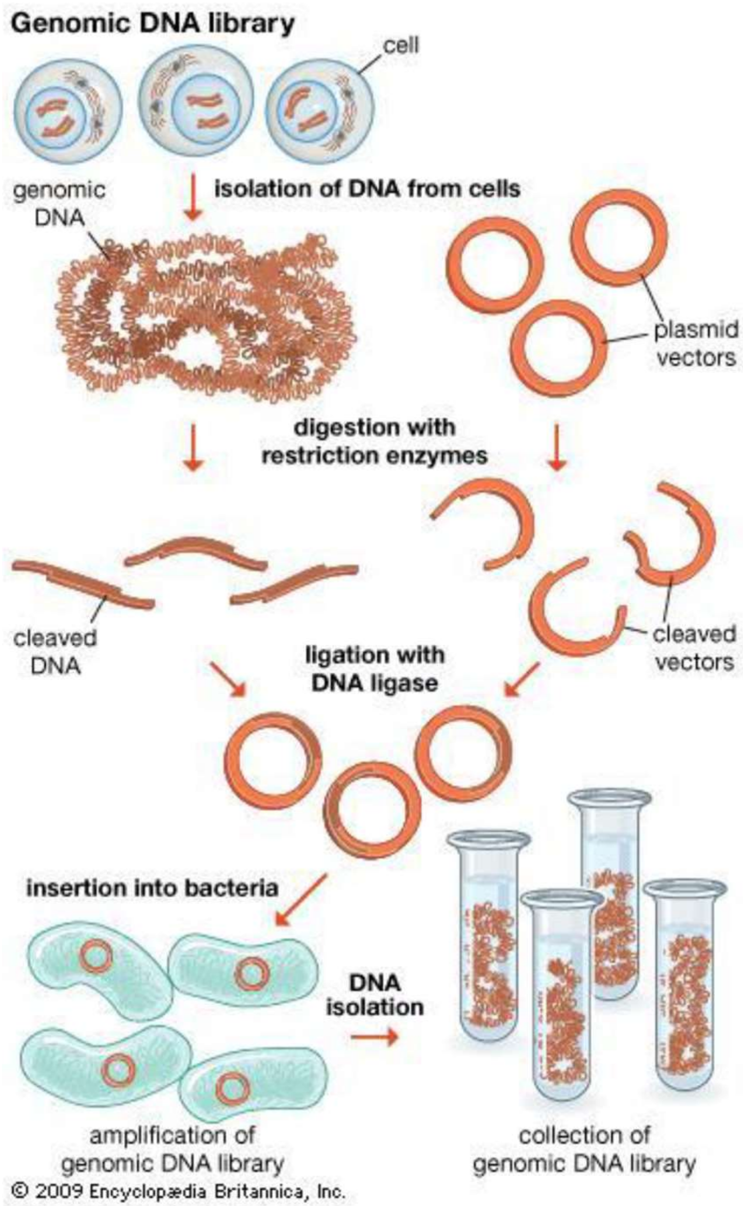
- The genes of all three types of HuIFN have been cloned in micro-organisms and expression obtained.

- HuIFN-β and γ produced in this manner lack the glycosylation present in the naturally occurring substances but this does not affect their specific activity.

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 HuIFN-α has been readily available.

Process


Genomic DNA library
cell
genomic DNA
isolation of DNA from cells
plasmid vectors
digestion with restriction enzymes
cleaved DNA
cleaved vectors
ligation with DNA ligase
insertion into bacteria
DNA isolation
amplification of genomic DNA library
collection of genomic DNA library
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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.

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.

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.

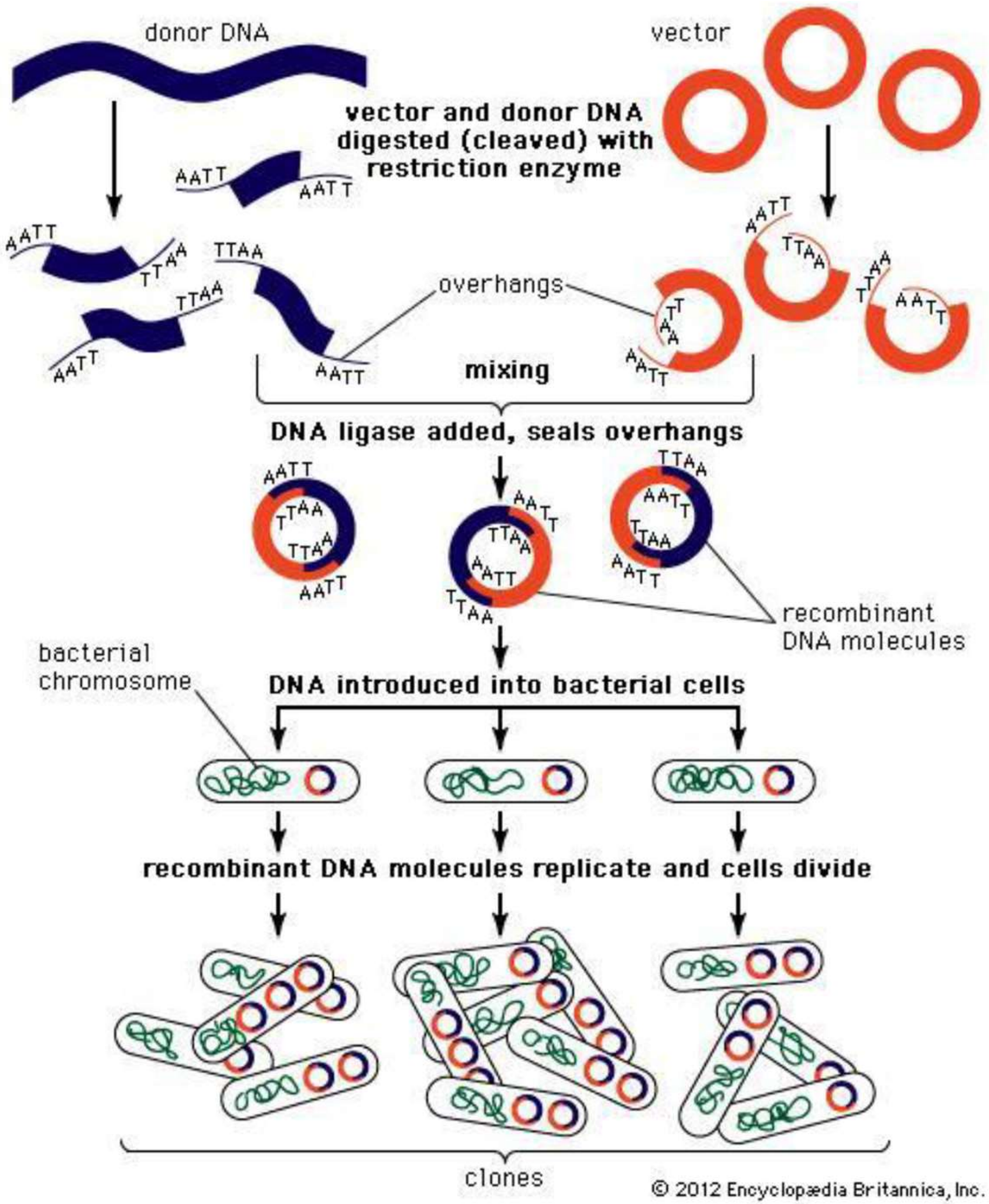
- **The mode of delivery of DNA vaccines include:** direct injection into muscle; use of vectors like adenovirus, retrovirus etc; *in-vitro* transfer of the gene into autologous cells and re-implantation of the same and particle gun delivery of the DNA.

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.

Process



donor DNA
vector
vector and donor DNA digested (cleaved) with restriction enzyme
overhangs
mixing
DNA ligase added, seals overhangs
recombinant DNA molecules
bacterial chromosome
DNA introduced into bacterial cells
recombinant DNA molecules replicate and cells divide
clones
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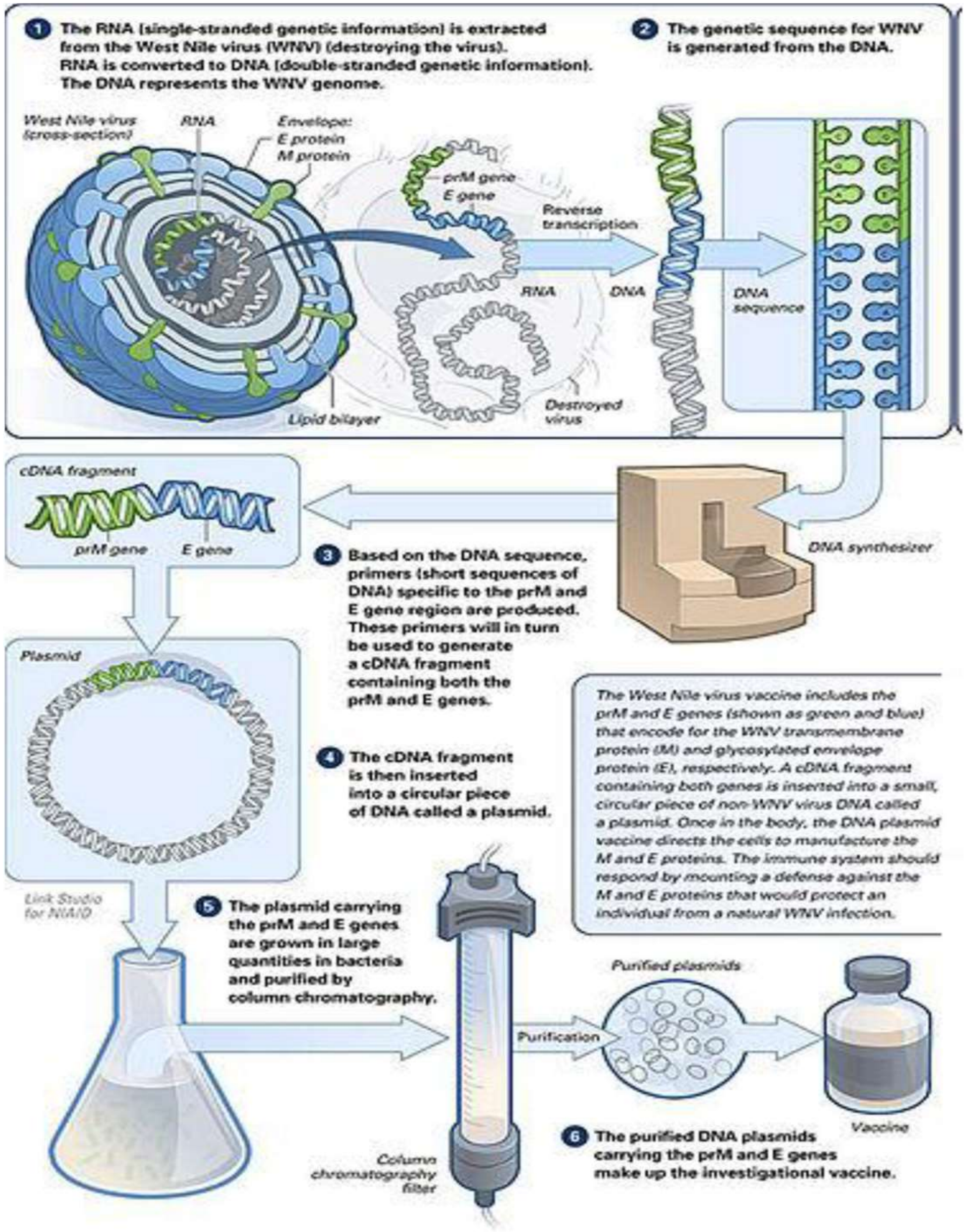
Process



1 The RNA (single-stranded genetic information) is extracted from the West Nile virus (WNV) (destroying the virus). RNA is converted to DNA (double-stranded genetic information). The DNA represents the WNV genome.
2 The genetic sequence for WNV is generated from the DNA.
West Nile virus (cross-section)
RNA
Envelope: E protein M protein
prM gene
E gene
Reverse transcription
RNA
DNA
DNA sequence
Lipid bilayer
Destroyed virus
cDNA fragment
prM gene
E gene
DNA synthesizer
3 Based on the DNA sequence, primers (short sequences of DNA) specific to the prM and E gene region are produced. These primers will in turn be used to generate a cDNA fragment containing both the prM and E genes.
Plasmid
4 The cDNA fragment is then inserted into a circular piece of DNA called a plasmid.
The West Nile virus vaccine includes the prM and E genes (shown as green and blue) that encode for the WNV transmembrane protein (M) and glycosylated envelope protein (E), respectively. A cDNA fragment containing both genes is inserted into a small, circular piece of non-WNV virus DNA called a plasmid. Once in the body, the DNA plasmid vaccine directs the cells to manufacture the M and E proteins. The immune system should respond by mounting a defense against the M and E proteins that would protect an individual from a natural WNV infection.
Link Studio for NIAID
5 The plasmid carrying the prM and E genes are grown in large quantities in bacteria and purified by column chromatography.
Purified plasmids
Purification
Vaccine
6 The purified DNA plasmids carrying the prM and E genes make up the investigational vaccine.
Column chromatography filter

THANK YOU