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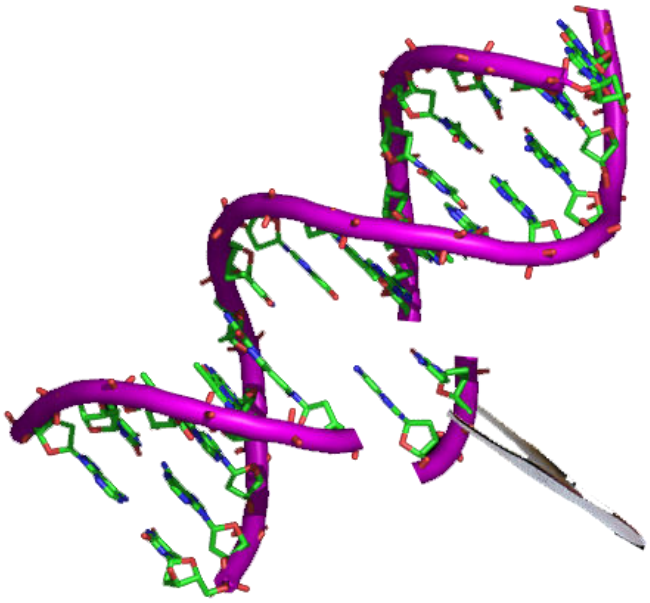
RECOMBINANT DNA TECHNOLOGY



Definition

Recombinant DNA Technology is defined as the technique used to create new combination of genetic material by joining DNA molecules from different organisms. The process involves cutting and joining of DNA at specific sites in order to produce chimerical DNA which can be expressed in the new host.

Biotechnology which is synonymous with **Genetic Engineering** or **Recombinant DNA Technology (rDNA)** is an industrial process that uses the scientific research on DNA for practical applications. Recombinant (rDNA) that is made

through the combination or insertion of one or more DNA strands. Recombinant DNA Technology deals in constructing new combination of genes in laboratory.

Genetic Engineering is used for creating multiple copies of genes and insertion of foreign genes into an organisms to give them new traits.

Introduction

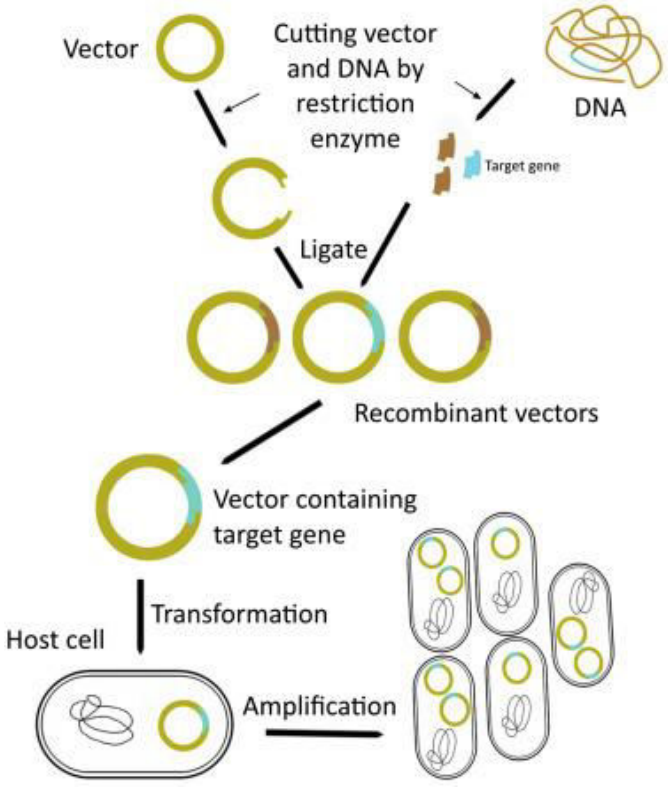
Recombinant DNA Technology or **Gene Cloning** is a new born discipline of science which aims to alter the heredity of a living organism.

Recombinant DNA is a form of artificial DNA which is made through the combination or insertion of one or more DNA strands therefore combining DNA sequences.

Genetic Engineering aims at manipulating the genes of an organism at will where techniques are applied on the biological system for the benefit of mankind.

Basic Principles of RDT

1 Generation of DNA fragments and selection of the desired piece of DNA.
2 Insertion of selected DNA into a cloning vector to create a recombinant vector.
3 Introduction of the recombinant vector into a host cell
4 Multiplication and selection of clones containing the recombinant molecule.
5 Expression of the foreign gene into the host to produce the desired product.



Steps of Recombinant DNA Technology

1. **Isolation/Synthesis of gene**
2. **Selection of a vector**
3. **Attachment of foreign gene to the vehicle**
4. **Transfer of recombinant DNA to the host**
5. **Expression of the transferred genome**

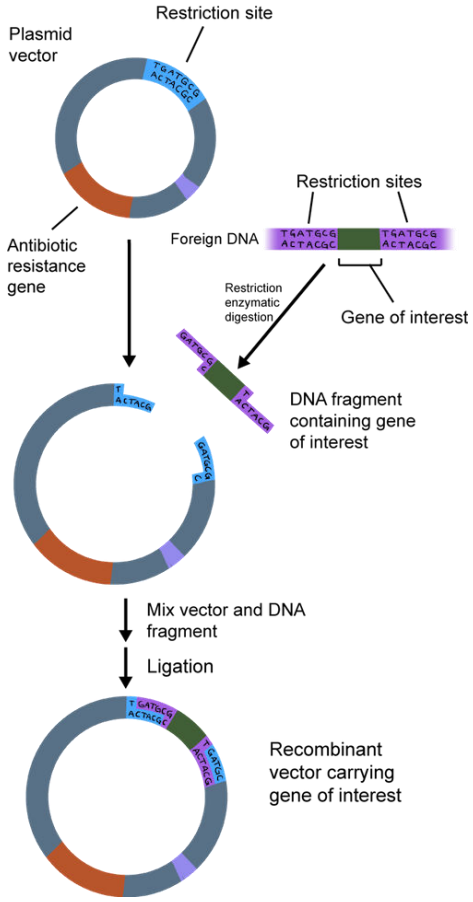


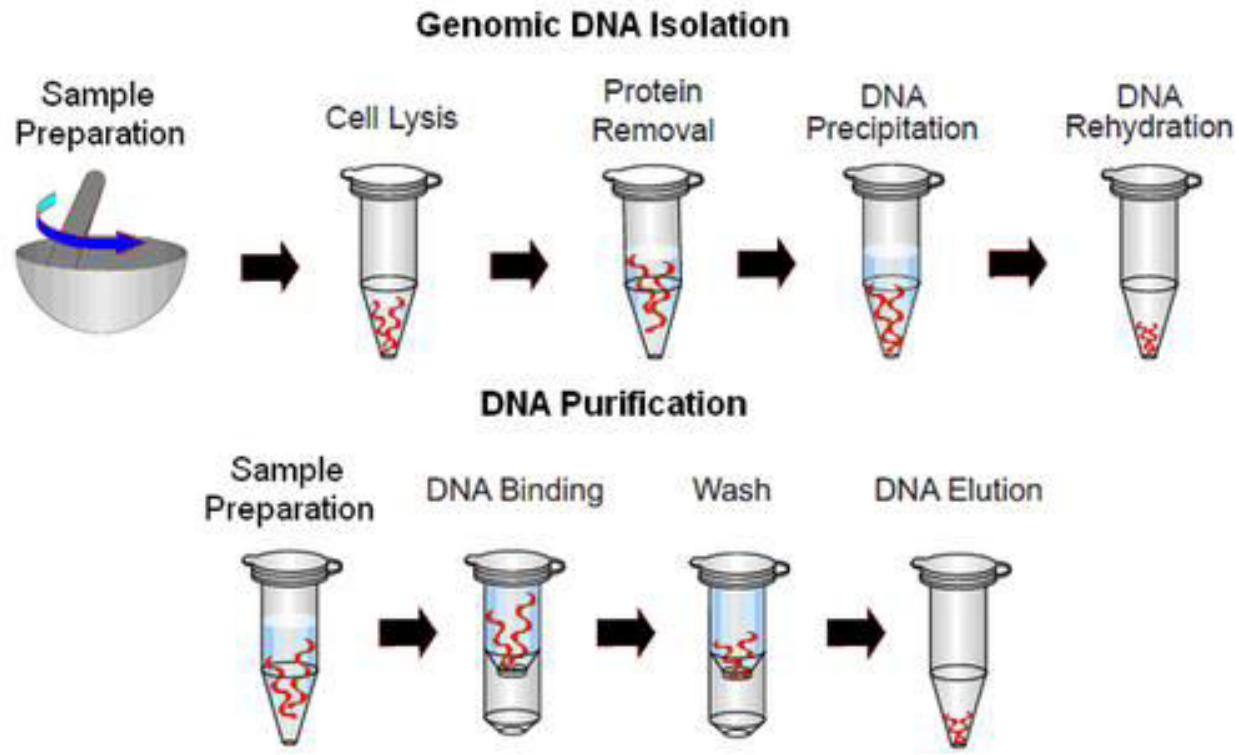
Fig. The steps for producing Recombinant DNA

STEP 1 ISOLATION OF GENE

Identification and isolation of the desired gene is required in order to generate new combination of genome. Significant progress has been made in the techniques for isolation of a variety of genes like rRNA , protein products, regulatory genes, promoter genes, etc. The first gene to be isolated was the lac- operon of E.coli (Shapiro et al , 1969)

Artificial (Organochemical) synthesis of gene

It was definitely a positive step of genetic engineering. H.G.Khorana (1970) was successful in synthesizing a double stranded DNA corresponding to the major Yeast alanyl tRNA.



STEP 2 SELECTION OF VECTOR

Vectors are the DNA molecules which can work as vehicle to carry foreign gene, either isolated or synthesized, into the host for multiplication or expression.

Vectors are selected according to the purpose and type of host in which it has to be cloned or expressed named as shuttle vector, cloning vector etc

Some common vectors are

1. Plasmids for prokaryotic host
2. Phages (Lambda phage, M13 phage)
3. Yeast plasmid for eukaryotic host
4. Ti and Ri plasmid for plants
5. Phagemids (pUC118, pUS119)
6. Transposons (P element of Drosophila, Ac & Ds of maize)Yeast Artificial Chromosome) & Mammalian Artificial Chromosome (YAC & MAC)
7. Artificial Plasmids (pBR322 , pBR327).

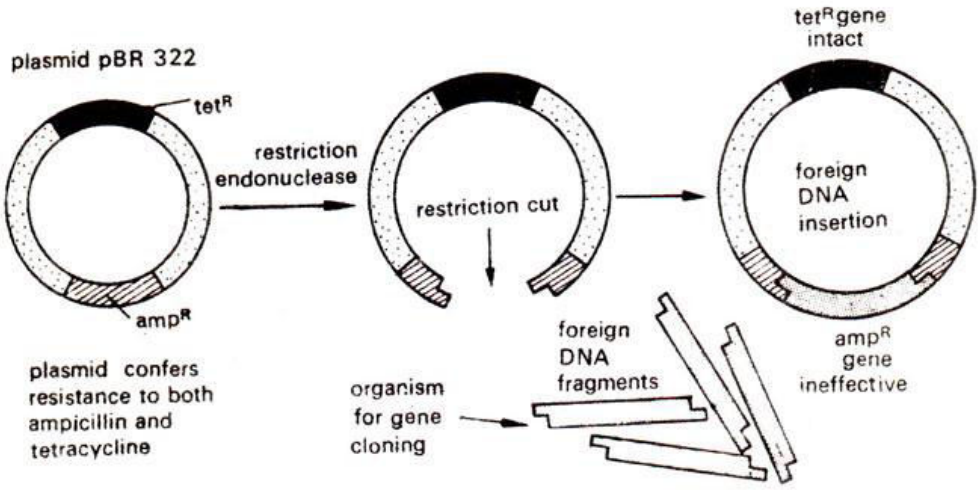
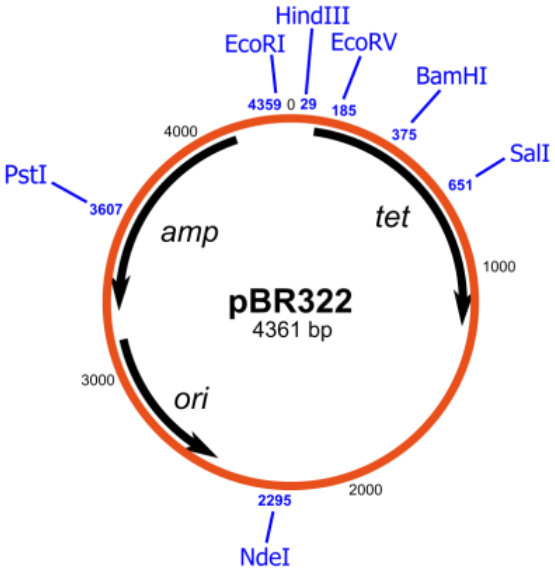


Fig: Plasmid being used as vector for gene transfer



STEP 3 Attachment of foreign gene to the vehicle

To produce recombinant DNA, site specific cutting and joining is the most important step. For cutting a duplex DNA at recognized site various **Restriction enzymes (RE)** has been isolated. Most suitable amongst them are **Type II Restriction Enzyme** which breaks the polynucleotide chain within recognized sequence for ex- Eco RI , Hae III, etc.

Restriction enzyme recognize a specific sequence of nucleotides and produce a double-stranded cut in the DNA. The recognition site is usually 4 to 8 bases.

Recognition site

```
5'...GAT|ATC...3'
3'...CTA|TAG...5'
```

A palindromic recognition site reads the same on the reverse strand as it does on the forward strand when both are read in the same orientation.

EcoRI digestion produces sticky ends
Derived from *Escherichia coli*

```
G|AATTC
CTTAA|G
```

Where as SmaI restriction cleavage produces blunt ends.:
Derived from *Serratia marcescens*

```
CCC|GGG
GGG|CCC
```

Different restriction enzymes produces DNA strands having different lengths of nucleotides but having same base pairs at the ends. The DNA generally are modified or methylated at restriction sites to protect themselves from own restriction endonucleases. On the basis of recognition site and modification sites RE are classified

Vector/ Vehicle and Passenger (Foreign) DNA can be joined together to produce heterologous DNA with the help of **Ligase enzyme**.

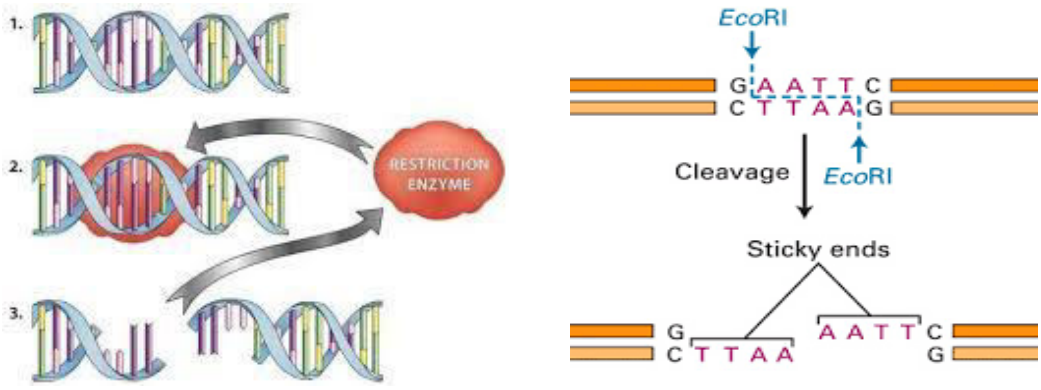


Fig. Cutting of duplex DNA with Eco RI to produce sticky ends (staggered cut)

STEP 4 Transfer of recombinant DNA to the host

After generation of recombinant DNA it is transferred into a suitable host either for multiplication (cloning) or for expression. Blackman et al (1977) have conducted a **shot gun experiment** to obtain an adequate number of E.coli clones carrying random fragments of Yeast DNA.

A rapid and simple technique for introducing cloned genes into a wide variety of microbial, plant and animal cell is by **Electroporation** were high voltage electric pulses can increase capability of plasma membrane to take in foreign molecules.

Transduction and **Transfection** are methods commonly used for transferring genetic material into bacterial/animal/plant cells.

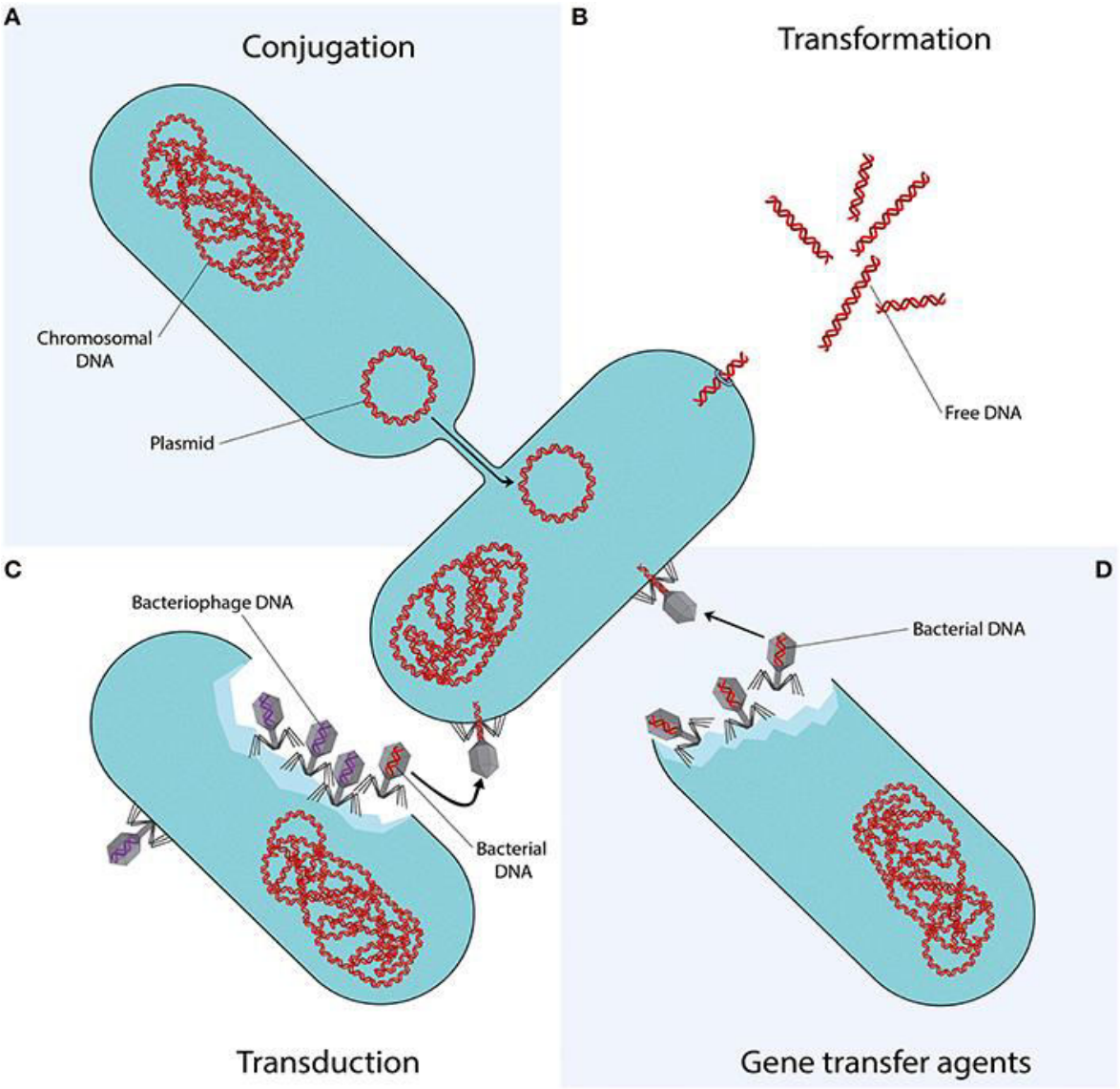


Fig: Transfer of gene into a bacterial host

STEP 5 Expression of the transferred genome

The control of replication and expression of the cloned DNA is one of the most important aspects of beneficial utilization of the Recombinant DNA Technology. The finding that the rat insulin gene can be transcribed and translated in E.coli cells open the possibility of large scale and economic production of insulin which is used as medicine for diabetic patients.

There are various applications of RDT like large scale production of alcohol, ascetic acid, enzymes , flavoring agents, etc.

Gene therapy, DNA fingerprinting, Diagnosis of molecular diseases are milestones of RDT.

In the field of Agriculture Tansgenic plants has increased tremendously the yield and quality of cereals, fruits and vegetables. BT brinjal, Golden rice, Delayed ripening tomato, Pomato , etc are few examples.

ROLE OF RDT IN HUMAN WELFARE

Recombinant DNA Technology has been applied in different fields for the welfare of mankind. Following are some of its applications:

1. **Application in Crop Improvement**

   Genetic Engineering has several potential applications in crop improvement.

   a. **Distant Hybridization** -- Due to advancement in GE it is now possible to transfer genes between distantly related species even genus, for example Raphanobrassica, Triticale, Pomato, etc.

   b. **Development of Transgenic plants** -- Genetically transformed plants which contain foreign genes are called transgenic plants. Resistance to disease, insects and pests, herbicides, drought, metal toxicity tolerance, etc can be achieved through this Technology. Some of the successful results are BT Brinjal, BT Cotton, Delayed ripening Tomato, etc.

   c. **Development of Root Nodules in Cereal Crops** -- The bacterial gene responsible for nitrogen fixation can be transferred now to cereal crops like wheat, maize, rice, barley etc which enables them to fix nitrogen like leguminous plants.

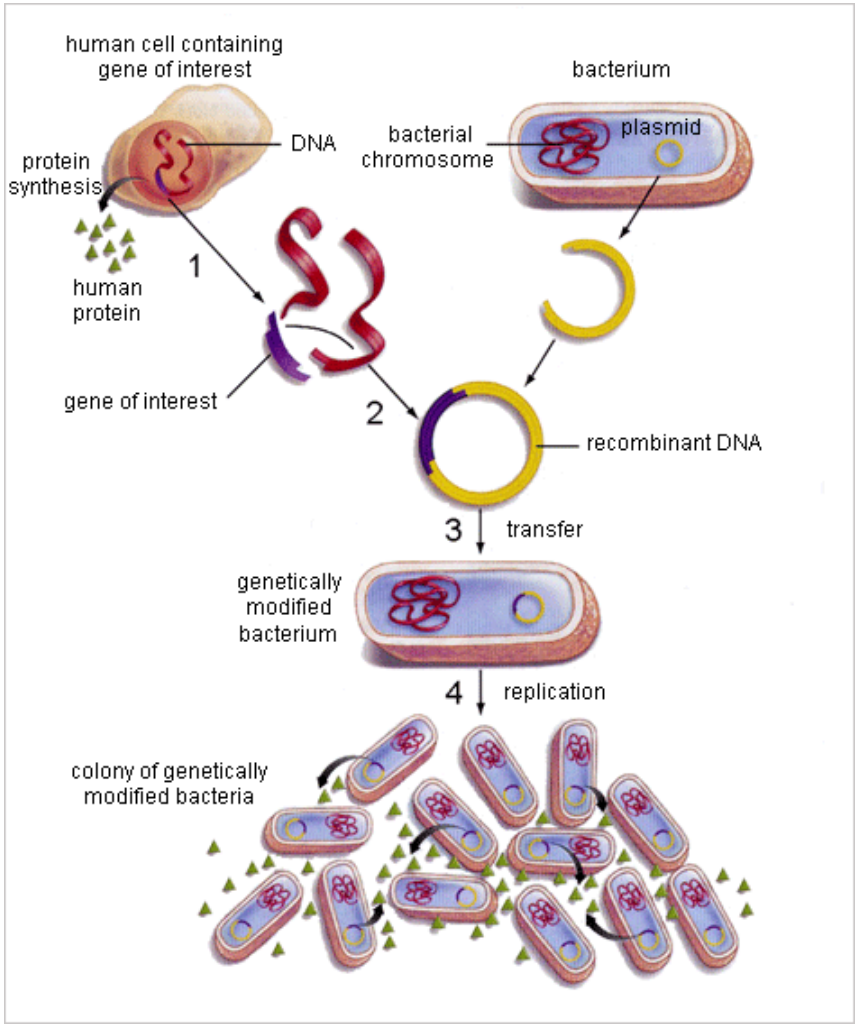
   d. **Grains with Improved Nutritional Characteristics** -- Rice grains do not contain carotene but Genetic engineering has led to develop a rice plant (Golden Rice) which produce yellow grains because of high carotene content. About 300 grams of this cooked rice a day can supply all the α-carotene a person needs.

2. **Application in Medicine**

Genetic Engineering plays an important role in the field of Medical Science

a. **Production of Antibiotics --** Penicillium and Streptomyces fungi has been used for mass production of famous antibiotic Penicillin and Streptomycin. Genetically efficient strains of these fungi have been developed to greatly increase the yield of these antibiotics.

b. **Production of Insulin** -- Insulin is a hormone, used by diabetics, was usually extracted from pancreas of cows and pigs. This insulin was slightly different in structure from human insulin. As a result, it leads to allergic reactions. It was expensive also. By using RDT Human gene for insulin production has been incorporated into bacterial DNA and such genetically engineered bacteria are used for large scale production of insulin. This insulin does not cause allergy. This has reduced the cost price of insulin as well.



c. **Production of Vaccines** --- A vaccine is a substance containing all or some part of a harmless version of pathogen that is introduced into the body to produce immunity against the pathogen. A DNA vaccine is a vaccine made from the DNA of a pathogen but does not have disease causing capability. This DNA vaccine is injected into a patient where it directs the synthesis of protein. The immunity develops against the protein. Researchers are working to develop DNA vaccines to prevent AIDS, malaria and cancers.

d. **Plantibodies** -- An antibody produced by genetically modified crops are known as plantibodies. Plants are transformed by introducing antibody genes from animals. This was done in 1989, with a mouse antibody made by tobacco plant. Some successful results are -- Hepatitis B vaccine, vaccine against HIV virus, Anthrax vaccine from tobacco plants (one acre of plant can produce 360 million doses in a year).

e. **Gene Therapy** -- Genetic Engineering may one day enable the medical Scientists to replace the defective genes responsible for hereditary diseases like Haemophilia, Phnylkeptonuria, etc with normal genes**.**

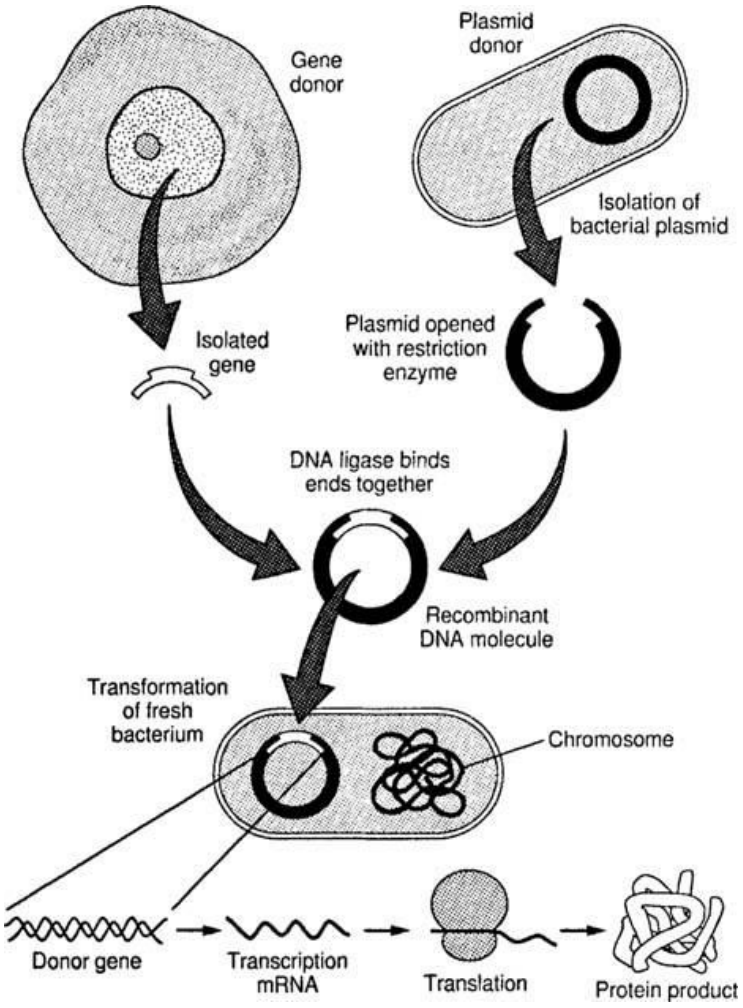
f. **Production of Enzymes** -- Some useful enzymes can be produced by Recombinant DNA Technology. The conversion of plasminogen to plasmin is activated by an enzyme called **Tissue Plasminogen Activator (TPA),** which is produced by lining of blood vessels. Heart attacks and many strokes are caused by blood clots. Treating these persons with this enzyme saved lifes. The discovery of TPA and its isolation from human tissues was used to make recombinant plasmid inserted into an expression vector, was transfected into E.coli. The transgenic bacteria made the protein in quantity and it soon became commercially available.

The following table shows some medically useful recombinant products and their applications:

Medically useful Recombinant Products	Applications
Human insulin	Treatment of insulin-dependent diabetes
Human growth hormone	Replacement of missing hormone in short stature people
Calcitonin	Treatment of rickets
Chronic gonadotropin	Treatment of infertilty
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with haemophilia A/B
Tissue Plasminogen Activator	Dissolving of blood clots after heart attacks and strokes
Erythropiotin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during kidney dialysis or side effects of AIDS patients treated by drugs.
Platelet derived growth factor	Stimulation of wound healing.
Interferon	Treatment of pathogenic viral infections, cancer.
Interleukins	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertussis, meningitis, etc.

3. Industrial Application

In industries, RDT helps in the production of chemical compounds of commercial importance. Improvement of existing fermentation processes and production of proteins from wastes. This can be achieved by developing more efficient strains of microorganisms. This is also known as crop improvement which ultimately increase the production of chemicals, medicines, enzymes, etc.



4. Generation of Transgenic Animals

The gene encoding the growth hormone somatotropin was first to be cloned successfully. The dairy farmers use Bovine Somatotropin (BST) worldwide as a supplement to their cows diets, increasing the animals milk production.

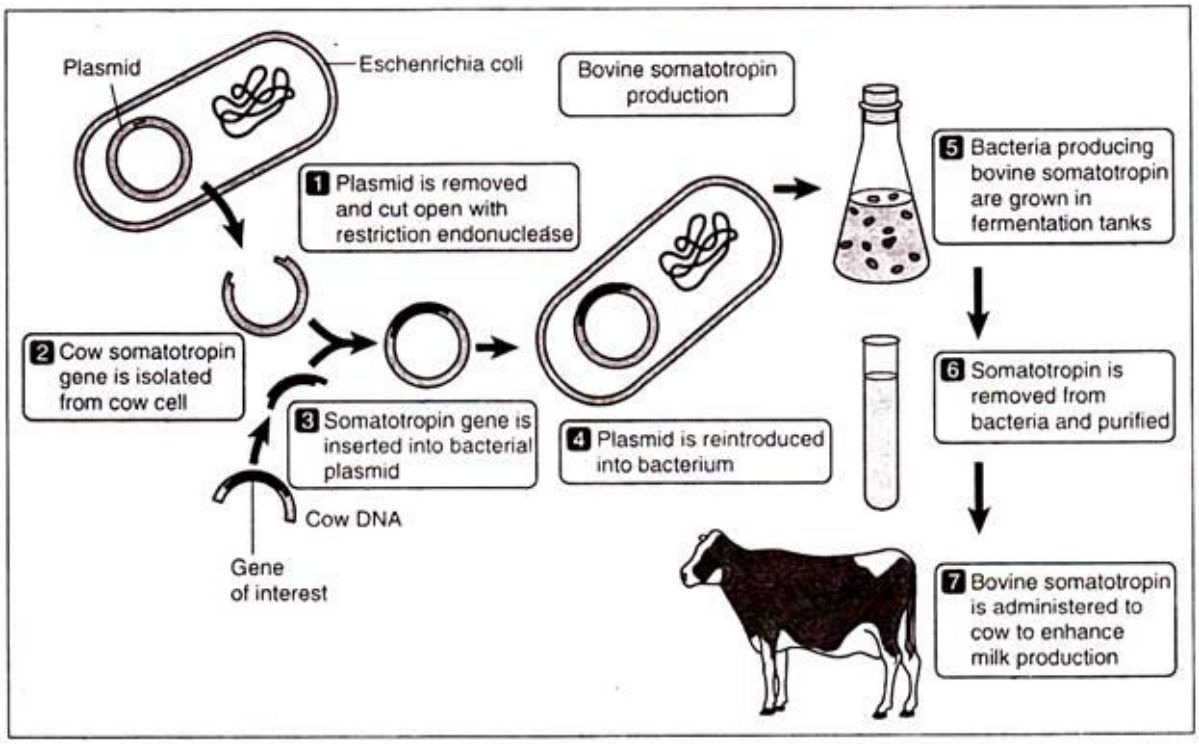


Fig. 2.9: The production of bovine somatotropin (BST) through genetic engineering. Although BST is functional, harmless, sanctioned by the FDA, much controversy exists over whether it is actually desirable

5. Application in Forensics

Genetic Engineering has an enormous impact on the field of forensic science. PCR (Polymerase Chain Reaction) or RFLP (Restriction Fragment Length Polymorphism) analysis can be used in criminal investigations. Both techniques produce a fingerprint or pattern of bands on gel. They use the non-coding regions of the human genome which can be used as a basis for discriminating between individuals.

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