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Topics

- Genomics
- Proteomics
- Tools of Genetic Engineering
- Beneficial features of transgenic animals

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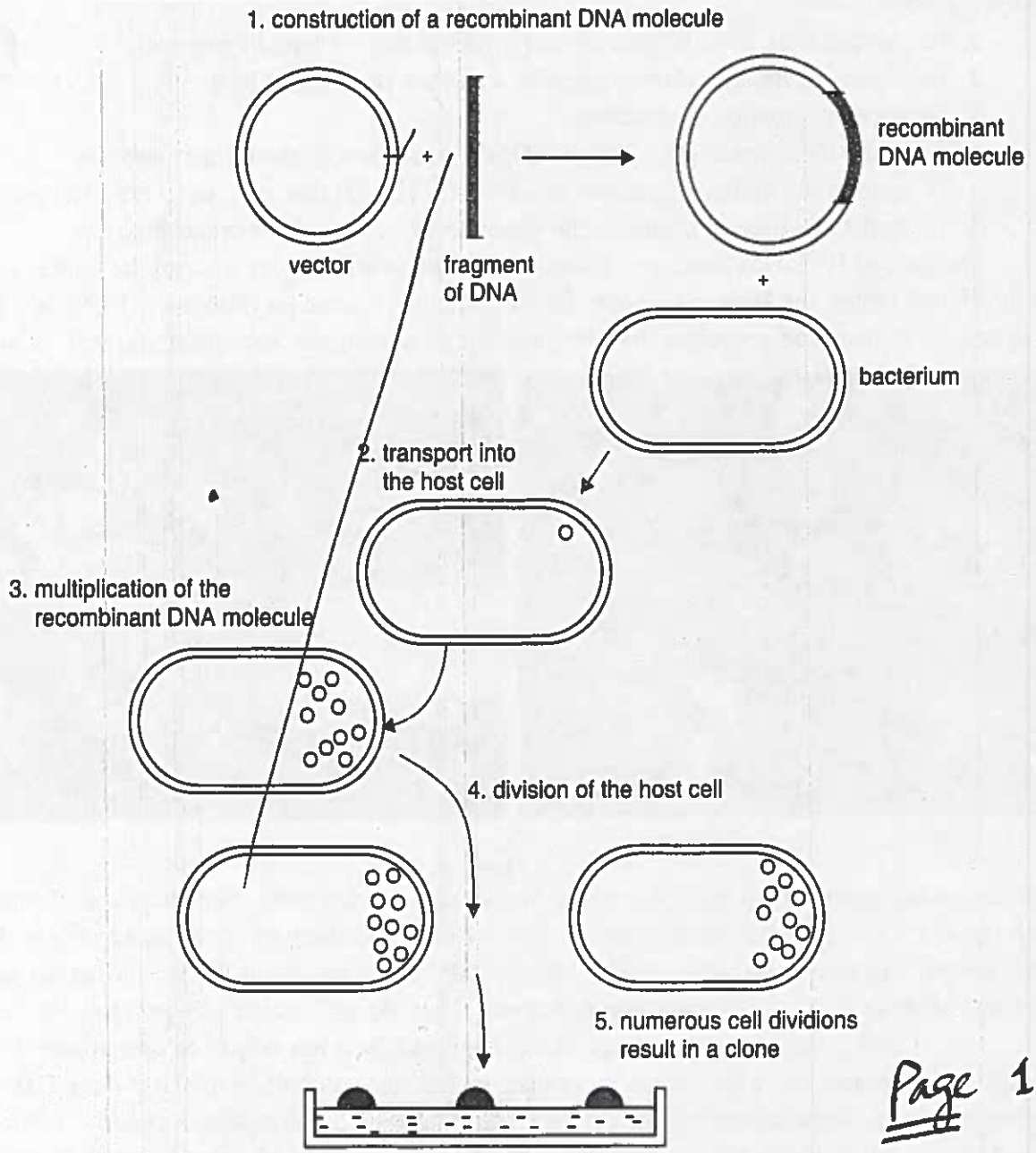

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Fig. 1.2. The basic events in a gene cloning experiment.

1.4. SCOPE OF GENETIC ENGINEERING

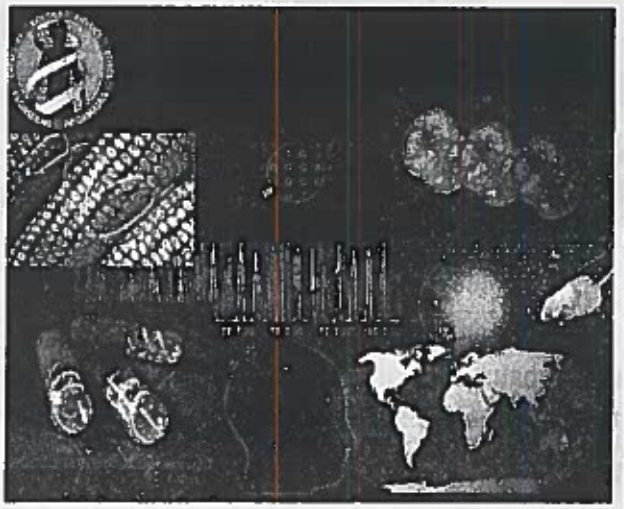
The current excitement about modern biotechnology is due to manner in which this science has pushed organisms beyond their natural abilities by genetic engineering and hybridoma technology. Nature has equipped every organism with the capacity to perform within an optimum or balanced system. Modern biotechnology has manipulated the genetic material of the organism by introducing foreign genes. The pupose is to push the organism to do things it did never before. Ability to genetically manipulate organisms right from viruses to mammals led to the **genomic revolution** – advocated as the **third technological revolution** following the *industrial revolution* and *computer revolution*. Genomic revolution has introduced following new fields:

1. Genomics. Computer-based study and designing of genome is called **genomics.** The term 'genomics' was coined recently in 1986 by **Thomas Roderick** to describe the scientific discipline of mapping, sequencing and analyzing genomes. **H.Winkler** in 1920 had coined the term **genome** to implicate the complete set of chromosomal and extra-chromosomal genes of an organism, a cell, an organelle or a virus. In fact, genomics began with the conception of the **Human Genome Project (HGP)** in the mid-1980's.

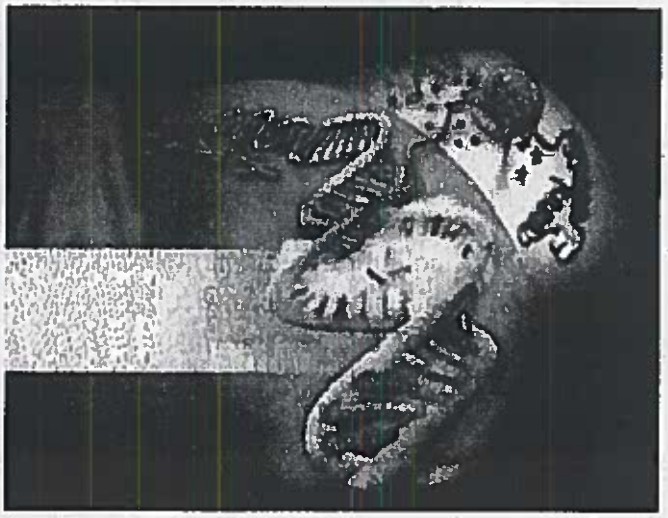
Human Genome Project (HGP). HGP is an international research programme. It has following objectives:

1. To construct the detailed genetic and physical map of human genome.
2. To determine the complete nucleotide sequence of human DNA.
3. To store information in database.
4. To locate the estimated 50,000 to 100,000 genes within the human genome.
5. To address the ethical, legal and social issues (ELSI) that may arise from the project.
6. To perform similar analysis on the genomes of several other organisms.

The goal of HGP was to sequence an estimated three billion base pairs of the human genome. In the United States, the Human Genome Project officially started on October 1, 1990, as a 15-year programme to map and sequence the complete set of human chromosomes, as well as, those of



Human Genome Project



Genomics

several model organisms. Almost the whole human genome has been sequenced and chromosome map has been developed in various laboratories world-wide through coordinated efforts. Human chromosome mapping was completed by March 2003. There are about 33,000 functional genes in humans. More than 97% genes are non-functional. They do not encode any polypeptide chain.

The 33,000 genes of human beings are on a microchip. It has helped to design specific drugs for genetic diseases, for which there is no clue so far. For example, a specific gene *-Her-2Neu-* overexpresses in breast cancer patients. A designed drug, called **Herceptin** is good for treatment of breast cancer. Thus, the field of genomis has helped the growth of pharmacological, toxicological and protein studies of animals. This trend initiated the origin of entirely new branches such as **pharmacogenomics, toxicogenomics** and **proteogenomics.**

Some achievements of genomics are the following:

1. Availability of recombinant proteins such as insulin (called **humulin**) made from bacteria (**biopharming**) has saved millions of pigs from being slaughtered.
2. Production of monoclonal antibodies by fusion of cancerous cells with B cells of immunized mice.
3. Production of **Golden rice** (a transgenic plant) by incorporating three genes required for production of vitamin A in Taipei rice.
4. Engineering of insect-resistant plants by the addition of gene for one of the insecticidal toxin of *Bacillus thuringiensis* (*Bt*) has improved the yield of cotton by seven percent per acre, and has reduced insecticide applications in the field.
5. Initiated the production of genetically engineered tomatoes, called Flavr savr.

II. Proteomics Study of all the proteins present on genome of an organism using computer is called **proteomics.** Thus, large scale characterization of the entire protein complement of cells, tissues and organisms is proteomics. The growth of proteomics is a direct result of advances made

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B. Immunology

1. National Institute of Immunology, New Delhi

2. Enzyme engineering, Immobile biocatalysts, Microbial fermenation and Bioprocess engineering, Institute of Microbial Technology, Chandigarh.

BTIS has made biomolecular database more accessible and more readily available for performing complex operations. A scientist sitting infront a computer terminal with biomolecular questions to address can proceed confidently knowing that they have at their disposal all the currently available data that bear on the question at hand and the ability to examine and manipulate that data. However, Indian planners must recognise that the need for computer education for biologists and other life biologists will become even more vital in the future (see **Ranga,** 2000).

7.4. PROTEOMICS

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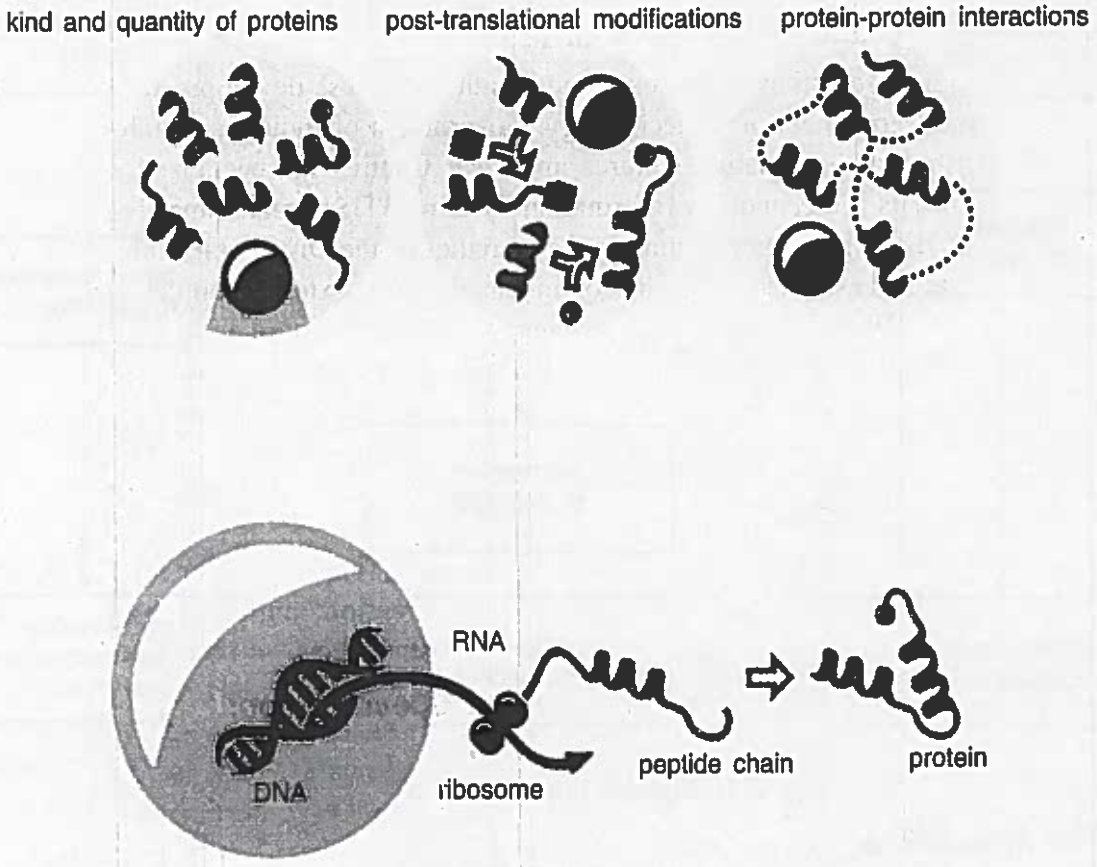
Proteomics is defined as the identification analysis and large-scale characterization of proteome (*i.e.*, total protein components) expressed by given cells, tissues and organs under the defined conditions. The main objectives of proteomics are (1) to characterise post- transcriptional modifications in proteins, and (2) to prepare three-dimensional map (*i.e.*, 3D) of a cell indicating the exact location of protein.



Proteomics.

Proteomics is the direct outcome of advancement made for nucleotide sequencing of different genomes in large scale. This helps to identify various proteins. Generation of information about protein is quite necessary, because, protein governs the phenotypic characters of the cells. Target drugs for many kinds of human diseases can only be prepared after understanding the protein modification and protein functions.

There are many areas of modern proteomics such as protein expression, protein structure, protein localization, protein-protein interaction, etc., (Fig. 7.4).

Relationship between Gene and Protein

In eukaryotes, **transcription** occurs inside the nucleus and **translation** takes place in the cytoplasm. DNA is transcribed under transcriptional regulation in pre-RNA by RNA polymerase II enzyme (Fig. 7.5). Inside the nucleus, pre-mRNAs undergo various post-transcriptional modifications to increase their stability such as capping at the 5'-end, polyadenylation at their 3'-OH end, and mRNA editing. The **introns** are spliced out by splicosomes through a process, called **splicing.** Then the mature mRNAs are transported from nucleus to cytoplasm for translation into protein on the ribosomes. Translation regulation of protein occurs in cytoplasm. Then, proteins undergo post-translational modifications, forming about 200 types of proteins. Often from a single gene, various forms of proteins are generated. This shows that is there no correlation between the number of proteins in an organism *i.e.*, the number of proteins are more than the number of genes.

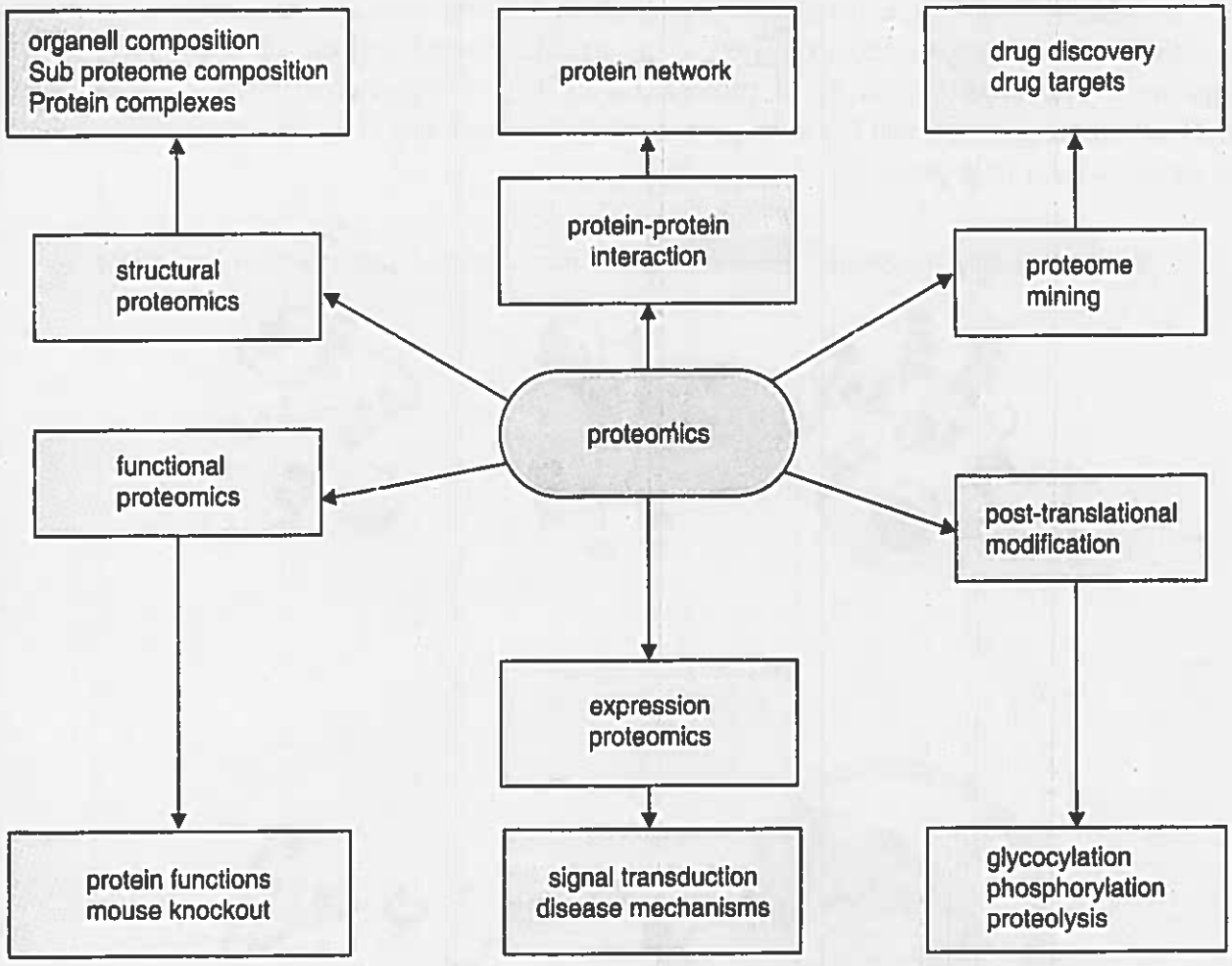


Fig. 7.4. Different branches of proteomics.

Types of Proteomics

Proteomics is divided into three main types: expression proteomics, structural proteomics and functional proteomics.

1. Expression proteomics: Expression proteomics is the quantitative study of protein expression between the samples which differ by some variables. A comparative study of the whole proteome between the samples can be done by using this approach. For example, a patient suffering from a mouth cancer develops a small tumor. Thus the tumor of cancer patient and similar tissues

from a normal person may be taken out and analysed for the protein expression by following different routes. Using techniques of high resolution protein separation and identification (*e.g.*, two-dimensional gel electrophoresis, isoelectric focusing, MALDI mass spectrometry, microarray techniques, etc.) under-expressed or over-expressed proteins in cancer patients and normal ones can be characterized and identified. An understanding about the formation of such tumor could be developed on the basis of proteins identified and compared between the two individuals.

2. Structural proteomics. The structural proteomics deals with the study of structure and nature of protein complexes present in a particular cell organelle. To fulfil this objective a specific subcellular organelle or all protein complexes are isolated. All proteins present in these complexes are identified and protein-protein interactions occurring between them are characterised. These studies lend support to assemble information about the structural topography of the cells and clues how certain proteins got expressed and gave unique characteristics to the cells.

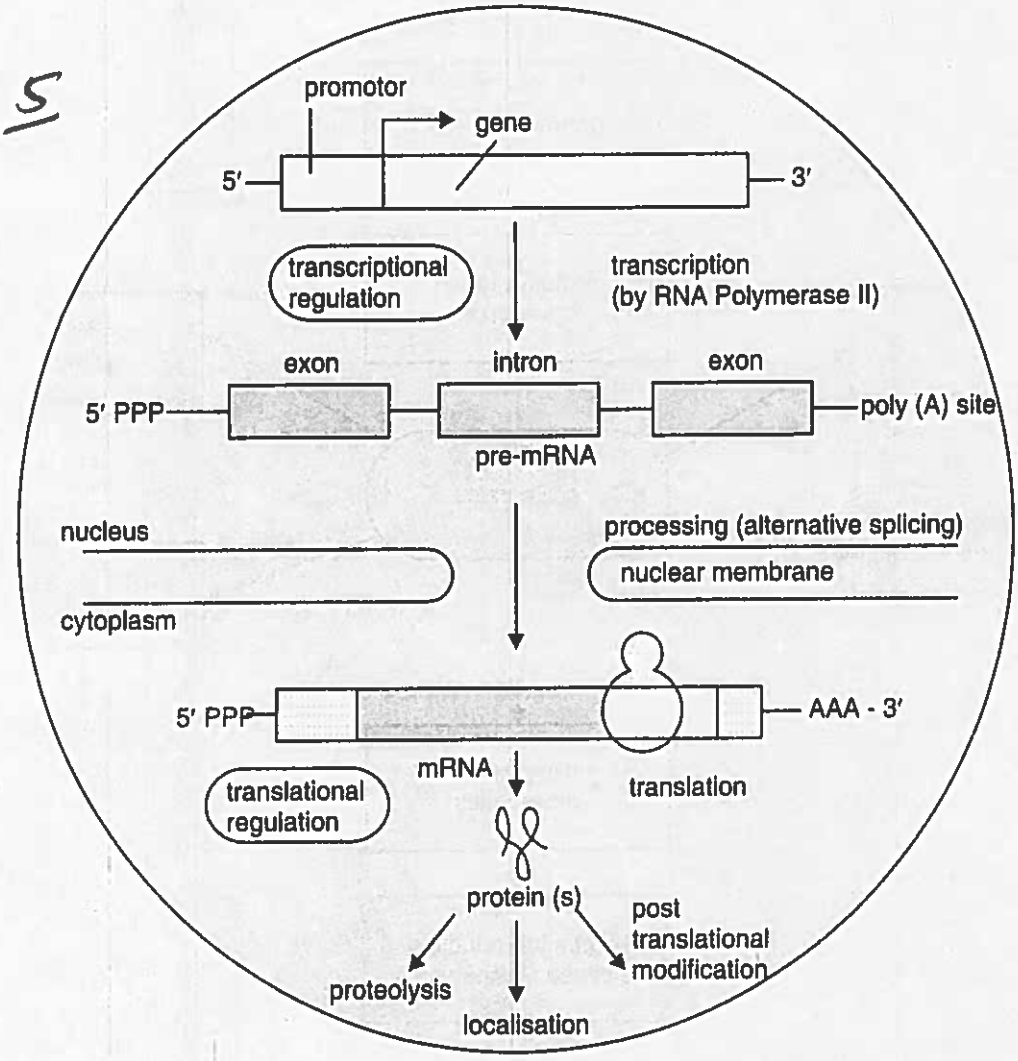


Fig. 7.5. Mechanism of production of multiple gene products from a single gene.

3. Functional proteomics. Functional proteomics comprises all proteomics approaches related to devising its functions. It is defined as the use of proteomics methods for analysis of properties of molecular networks formed in a living cell. In this study, molecules are identified which take part in such networks. Recently, some new proteins have been discovered which transport the important molecules from nucleus to cytoplasm and from cytoplasm to nucleus. This functional proteomics in rather a complex process where function of a molecule is found out in the molecular network of the cell.

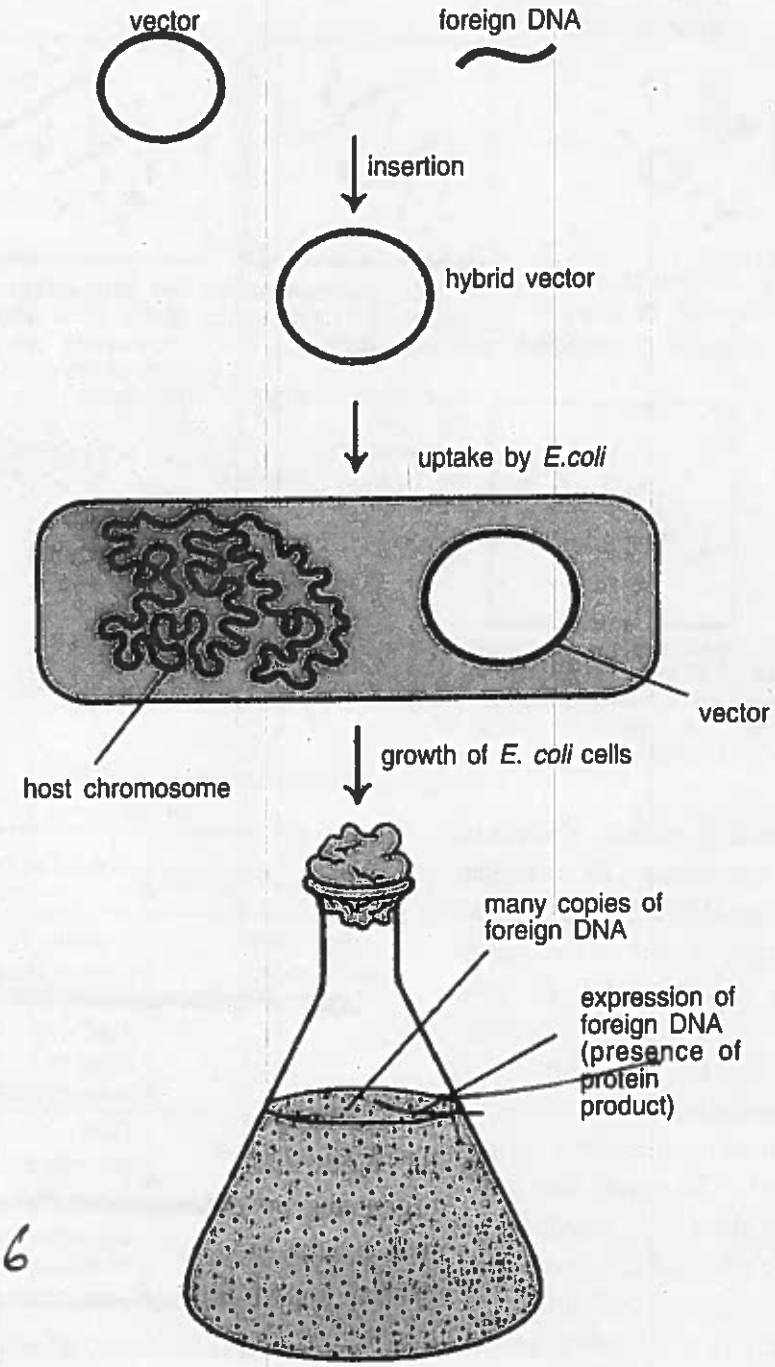


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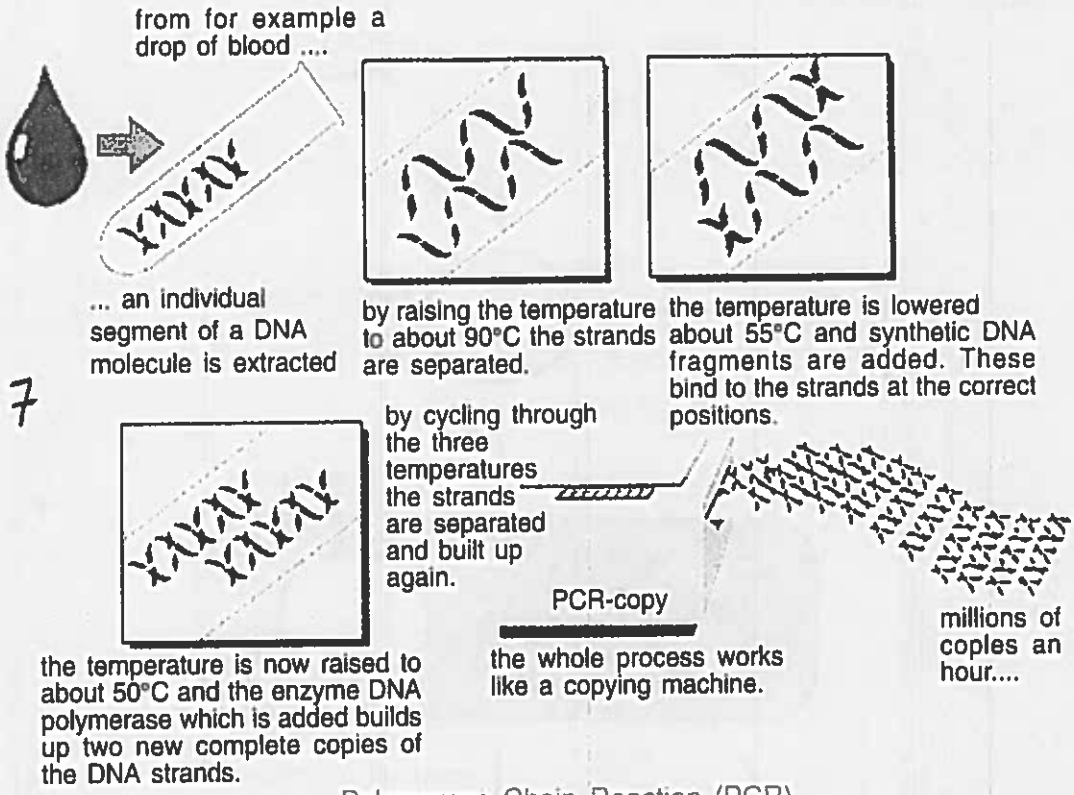
Fig. 4.1. Overview of recombinant DNA techniques. A hybrid vector is created, containing an insert of foreign DNA. The vector is then inserted into a host organism. Replication of the host results in many copies of the foreign DNA and, if the gene is expressed, quantities of gene products (All DNA shown is double-stranded) (after Tamarin, 2002).

4.1. TOOLS OF GENETIC ENGINEERING

There are various biological tools which are used to carry out manipulation of genetic materials and cells as well. Some of them have been described as follows :

1. **Enzymes** such as exonucleases, endonucleaes, restriction enzymes (=restriction endonucleases), SI enzymes (to change cohesive ends of single– stranded DNA fragments into blunt ends), DNA ligases, alkaline phosphatase, reverse transcriptase, DNA polymerases.

2. **Foreign DNA/Passenger DNA.** It is a fragment of DNA molecule which is enzymatically isolated and cloned. The gene is identified on a genome and pulled out from it either before or after cloning. The cloned foreign DNA fragment expresses normally as in parent cell.



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Polymerase Chain Reaction (PCR).

3. Cloning vectors. Vectors or vehicle DNA are those DNA that can carry a foreign DNA fragment when inserted into it. Based on the nature and sources, the vectors are grouped into bacterial plasmids, bacteriophages, cosmids and minichromosomes.

Plasmids have various curious properties : 1. The genes they carry may not be absolutely essential for life and so a plasmid can sometimes leave one bacterial cell and enter another, thereby transferring genetic traits between cells. 2. The plasmid can reproduce itself inside the bacterium independently of the main bacterial DNA. 3. A plasmid can sometimes fuse with the main DNA and later on can depart from the main genome, but in such a manner as to drag a piece of the main DNA with it. Nature seems to have evolved plasmid as an efficient way of exchanging gene between bacterial cells (see **Nossal** and **Coppel,** 1989).

4. DNA library. The first step in a gene cloning experiment is to obtain a DNA carrying the specific gene(s) that we are interesting in cloning. There are two main sources from which DNA is

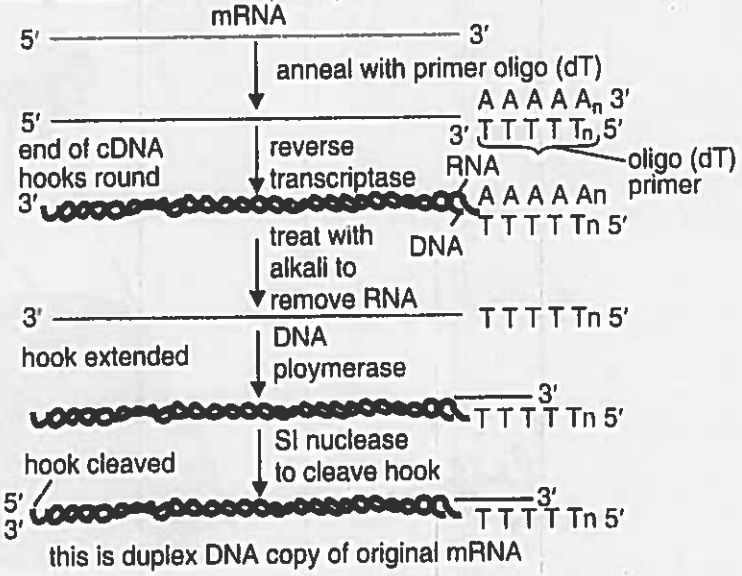
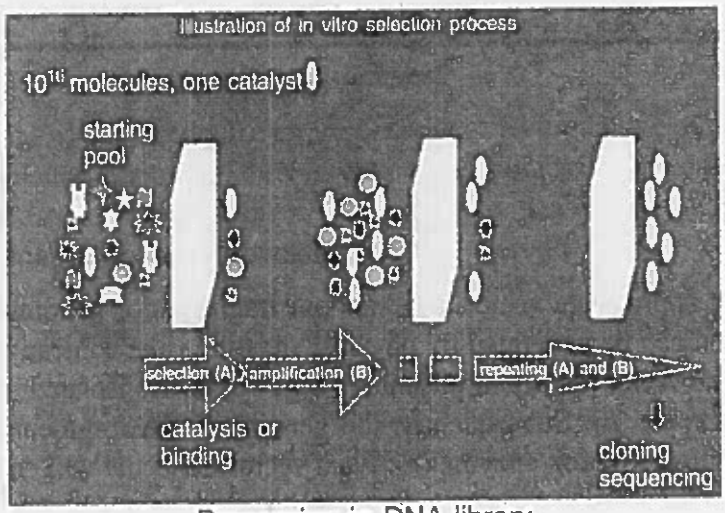


Fig. 4.2. Synthesis of cDNA from mRNA, using reverse transcriptase enzyme.



Processing in DNA library.

obtained for cloning experiments designed to identify an unknown gene – genomic DNA and cDNA (*i.e.*, complementary or copy DNA to mRNA). These DNA sources are used in the construction of two kinds of DNA libraries–genomic library and cDNA library.

(*i*) **Genomic library.** A genomic library is a collection of clones representing the complete genome of an organism. This means that the entire genome of an organism is represented as a set of DNA fragments inserted or cloned into a vector molecule that can be propagated in a suitable host. As shown in Figure 57.3 and Figure 57.4 to prepare a genomic library, the total genomic DNA is isolated from a tissue (*a*) and is fragmented into random pieces of suitable size for insertion into

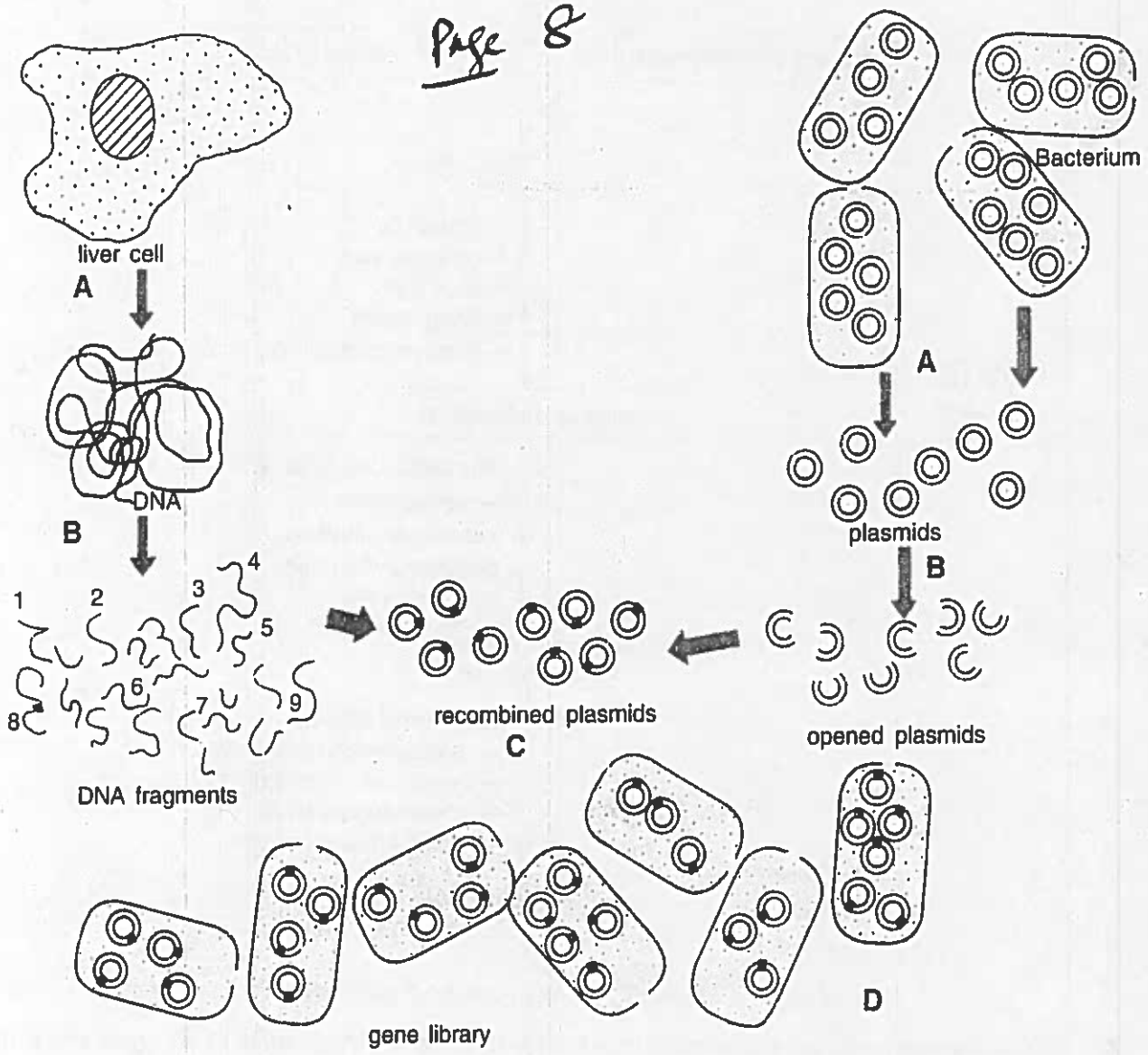


Fig. 4.3. Method of making a gene or genomic library.

an appropriate vector. The random fragments of genomic DNA are designated by numbers 1 to 9 and can be generated by physical shearing (like sonication) or partial restriction enzyme digestion. The vector (shown as plasmid in Fig. 4.3) is also isolated from a bacterium and (a) cut with restriction enzyme (*b*): Subsequently, the genomic DNA fragments are inserted into the suitable vector cut with restriction enzyme (*c*): Each vector molecule will contain a different fragment of DNA and the

recombinant DNA molecules thus generated are introduced into an appropriate host such as bacterial cells or phage particles (*d*).

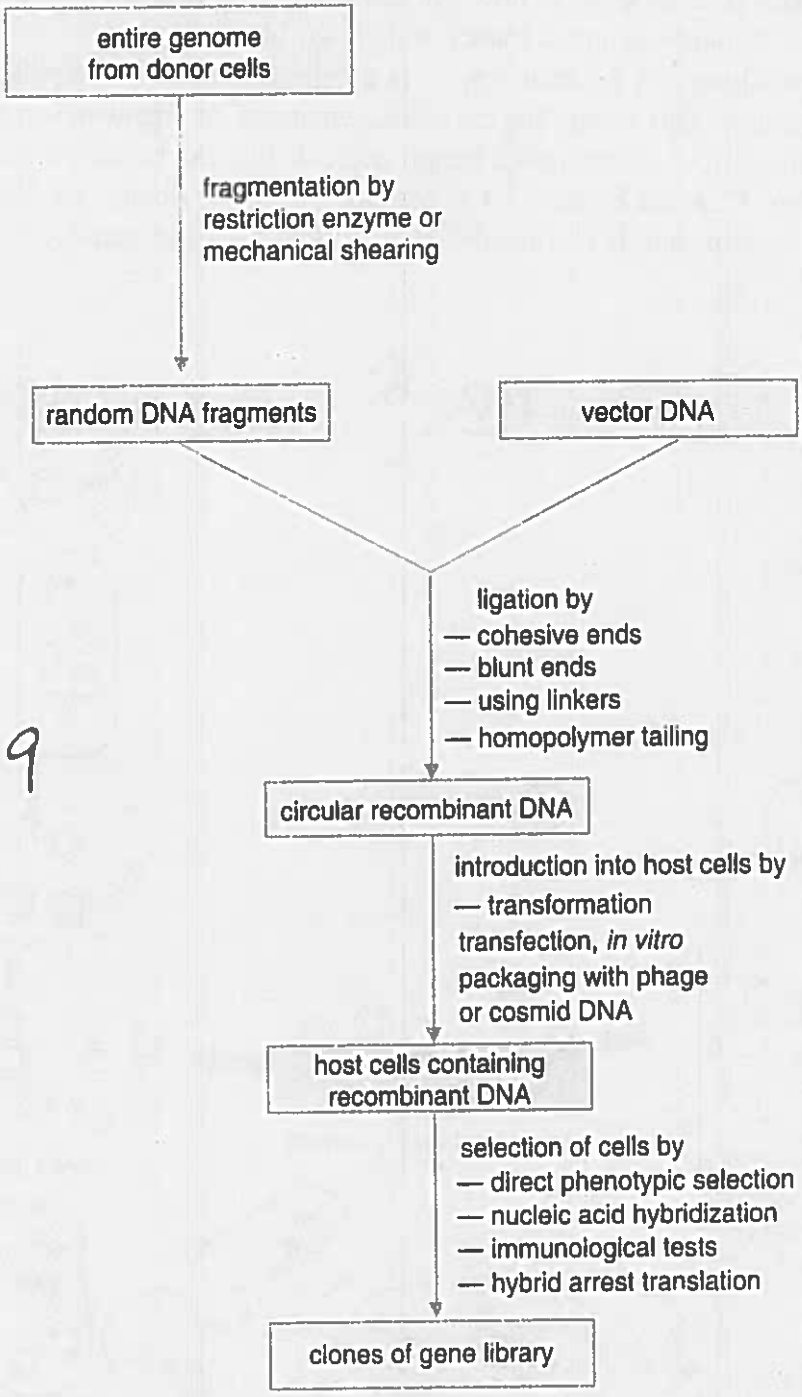


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Fig. 4.4. Methods of construction of gene library.

(*ii*) **cDNA library.** Although a genomic library represents the entire genome of an organism, it may not be useful in case of eukaryotic organisms. Genomic DNA of eukaryotes contains non-coding DNA such as introns, control regions and repetitive sequences. Therefore, another library called **cDNA library** can be made using messenger RNA (mRNA) as the starting material (Fig. 4.2). This library has two major advantages over a genomic library. Firstly, it represents only those genes that are being expressed by a particular cell or under a specific condition. Secondly, since mRNA lacks introns, this library would represent only the coding sequence of the genes. However, it is not possible to directly clone the mRNA since the RNA is unstable. Hence, it is converted into its DNA copy (cDNA) before cloning into a suitable vector. Such a library made from cDNA or

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complementary DNA is called a cDNA library.

The construction of a cDNA library begins with the isolation of mRNA from the cell type or tissue of interest. These mRNA molecules are reverse transcribed into cDNA molecules by the enzyme reverse transcriptase. By enzymatic methods, these single-stranded cDNA molecules are then converted into double-stranded molecules (Fig. 4.2), which are then inserted into a vector (Fig. 4.3). These recombinant DNA molecules are finally introduced into a suitable host (Fig. 4.4).

There are following methods by which recombinant DNA can be introduced into host cells; transformation, transfection, electroporation, microinjection, gene gun, or by the help of the bacterium *Agrobacterium tumefaciens* (see chapter 5).

5. **Molecular probes.** A probe is either a radioactive labelled (^{32}P) or non-radioactive labelled (*viz.*, biotin or digoxigenin), single– stranded nucleic acid (20–40 nucleotide long)g with a sequence complementary to at least one part of the desired DNA. The probe may be partially pure mRNA, a chemically synthesized oligonucleotide or a related gene which identifies the corresponding recombinant DNA. DNA/RNA probes have been commercially exploited in the diagnosis of infectious diseases, a variety of microbiological tests, identification of food contaminants and in forensic tests (*e.g.*, fingerprinting of murderers and rapists). Antibodies are also occasionally used as probes to recognize specific protein sequences (see **Dubey**, 1995).

4.2. CERTAIN GENERAL TECHNIQUES OF GENETIC ENGINEERING

The opening move of the genetic engineer is to break the DNA up into small, manageable bits, each containing one or just a few genes. Each little bit, one at a time, is stitched into a special, virus like piece of DNA gifted with the ability for self-replication. These virus-like, recombined DNA molecules now invade rapidly-dividing host cells, again one at a time. Each host cell (*e.g.*, a bacterium or yeast) thereby becomes a factory for one pure gene: Clever tricks allow the genetic engineer to pick out the host cell carrying the gene wanted for that particular experiment. By isolating that one special cell and growing it up to any desired quantity, the one desired gene (or its protein product) can be obtained. Now, let us consider some of the general methods of genetic engineering as follows :

1. Isolation and Use of Restriction Enzymes

Recombinant DNA technology makes a frequent use of restriction endonucleases which cut the DNA double helix in very precise ways. They have the capacity to recognize specific base sequences on DNA and then to cut each strand at a given place. These enzymes are called **restriction enzymes** because they restrict infection of bacteria by certain viruses (*i.e.*, bacteriophages), by degrading the viral DNA without affecting the bacterial DNA. Thus, their function in the bacterial cell is to destroy foreign DNA that might enter the cell. The restriction enzyme recognizes the foreign DNA and cuts it at several sites along the molecule. Each bacterium has its own unique restriction enzymes and each enzyme recognizes only one type of sequence. As already described elsewhere, the DNA sequences recognized by restriction enzymes are called **palindromes.** Palindromes are the base sequences that read the same on the two strands but in opposite directions. For example, if the sequence on one strand is GAATTC read in $5' \rightarrow 3'$ direction, the sequence on the opposite strand is CTTAAG read in the $3' \rightarrow 5'$ direction, but when both strands are read in the $5' \rightarrow 3'$ direction the sequence is the same. The palindrome appears accordingly —

5′ GAATTC 3′

3′ CTTAAG 5′

In addition, there is a point of symmetry within the palindrome. In our example, this point is in the centre between the AT/AT. The value of restriction enzymes is that they make cuts in the

2. Transgenic Animals :

When an animal carries a foreign gene, then it is called a transgenic animal. This is accomplished through the recombinant DNA technology. The foreign gene (transgene) introduced into the host is in addition to its normal genome. The transgene is expressed correctly in the host.

Beneficial features of Transgenic animals :

1. Pharmaceutically important drugs and proteins are produced in large quantity in transgenic cattle.
2. *Xenotransplanation* : Transgenic pigs produce transplant organs like heart, kidney, pancreases etc, which can be transplanted in humans known as **xenotransplantation.**
3. *Biochemical products* : Transgenic cattle produce many biochemical products like insulin, human growth hormone, blood anti-clotting factor etc. Transgenic milk may be used for treatment of different diseases like cystic fibrosis, phenylketonuria etc.
4. Production of human cell lines for curing genetic disorders like haemophilia, Alzheimer, thalassemia etc.
5. *Human gene therapy* : The defective gene copy of a person can be replaced by a normal copy the gene. A transgenic calf produces a substance that promotes the growth of red blood cells in human.
6. *More milk, meat and wool* : Transgenic cows produce more milk or milk with less lactose or cholesterol. Pigs, goats, chicken produce more meat. Transgenic sheep produce more wool.
7. *Production of disease-resistant cattle* - Influenza resistant pigs have been produced. Attempts are going on to produce other disease -resistant animals.
8. *Breeding of animals* : Through genetic engineering breeding of animals becomes easier and takes less time. Livestock can also be improved genetically.
9. *Industrial applications* : Transgenic animals have a number of industrial applications. Transgenic goat containing transgene from spider could produce small silk strands along with milk which can be extracted. These silk strands are weaved to produce threads which are light, tough and flexible and are used in military uniforms, medical microsutures, tennis racket strings etc.