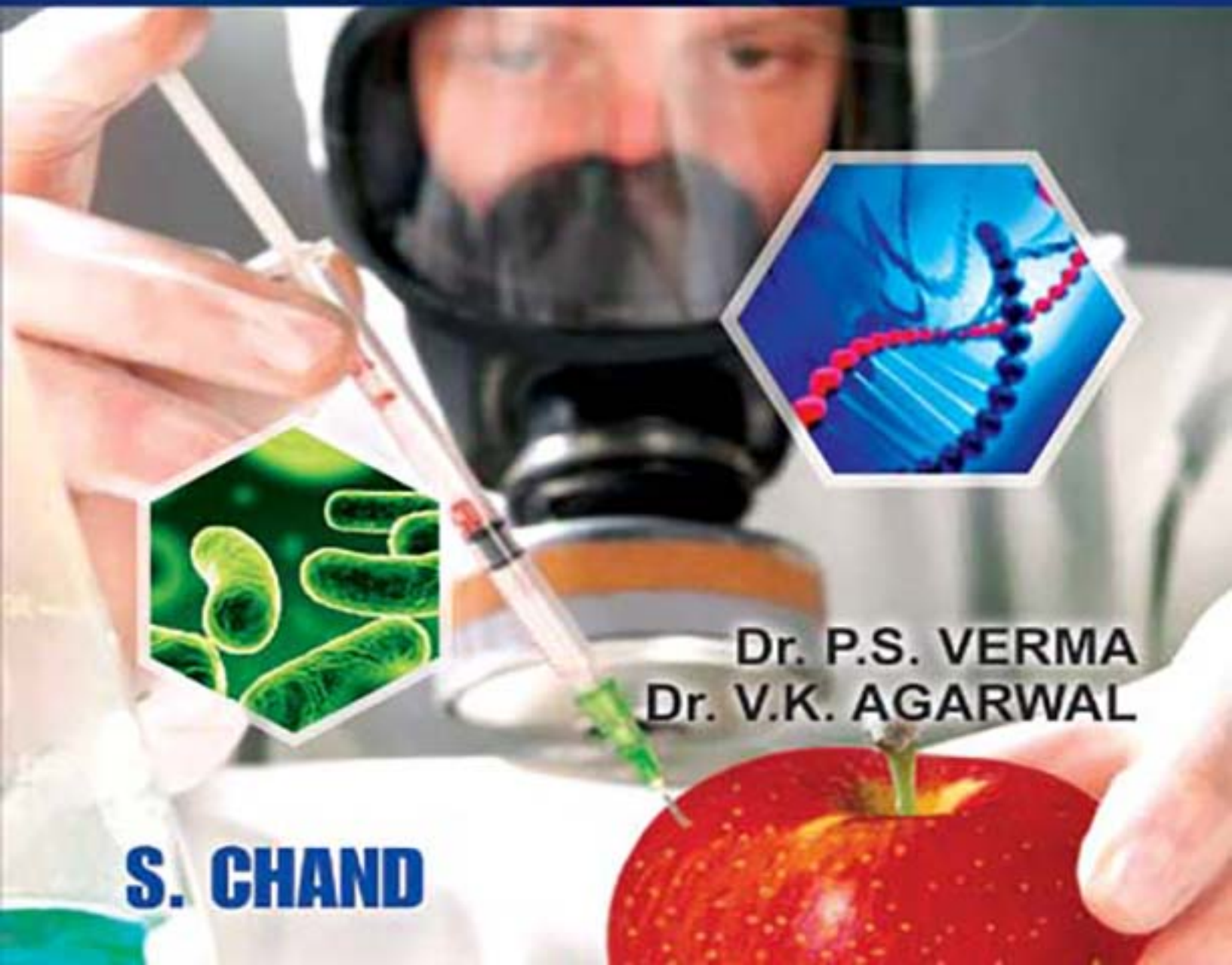
Genetic Engineering

For B.Sc., M.Sc. and BE/ B.Tech. Students



Dr. P.S. VERMA
Dr. V.K. AGARWAL

S. CHAND

Multicolour Edition

Genetic Engineering

For B.Sc., and M.Sc., classes of all Indian Universities
and as per UGC Model Curriculum

Dr. P.S. Verma
M.Sc., Ph.D., F.E.S.I., F.A.Z.
Reader (Retd.),
Department of Zoology
Meerut College, Meerut

Dr. V.K. Agarwal
M.Sc., Ph.D.,
Head and Reader,
Department of Zoology
Meerut College, Meerut

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PREFACE

Genetic Engineering is a sub-discipline of biotechnology. According to **Prof. Kannan** (2003) in Genetic Engineering, one adds, substracts, multiplies, or divides a gene, which in turn, changes the levels of a protein which may in turn changes the levels of carbohydrates and lipids. A classical biotechnologist first used nature's bio-factories (cells) to make products such as curd, ethanol, penicillin, insulin, etc. Modern biotechnology on the contrary is making cells, doing jobs which they have never done before. An instance being bacteria producing human insulin (the humulin).

The textbook of **Genetic Engineering** contains 7 chapters and about 150 pages. The text of the book has been kept quite simple, lucid and illustrative. By the help of ample figures, many tables, information-packed boxes, and coloured photographs, authors have tried to serve a balanced account of Genetic Engineering.

This textbook of **Genetic Engineering** has been developed for B.Sc. students of all Indian Universities. But it will also serve the purpose of the students of B.Sc. (Hons.), M.Sc. Zoology, Botany, Microbiology and Biotechnology. Students preparing for Medical entrance exams, I.F.S., P.C.S. and I.A.S., etc. would also be benefited by this book.

This book is the outcome of sincere and combined tireless efforts of the authors and the editorial team of Mr. Shishir Bhatnagar, Dr. Swati Lohani and Mr. Pradeep Joshi (of DTP Unit) of S.Chand and Co. Ltd. Title of the book is the creation of Mr. Gopal.

Authors wish to express their sincere thanks to Mrs. Nirmala Gupta, Chairperson and Managing Director, Mr. Himanshu Gupta, Director, Mr. Amit Gupta, CEO and Mr. Navin Joshi, Vice-President (Publishing) of S.Chand and Company for their wholehearted cooperation to bring out the multicolour edition of this book.

Constructive suggestions for further improvement of the book will be always welcomed and entertained.

Dr. P. S. VERMA
"PREETI"
121, New Mohanpuri Colony
Meerut- 250 001

Dr. V.K. AGARWAL
"Neelkanth"
566, Brahmpuri
(Sharda Road)
Meerut- 250 002

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Prologue

Genetic engineering is the artificial synthesis, isolation, modification, combination, addition and repair of the genetic material (DNA) to alter the phenotype of the host organism (*e.g. E.coli,* yeast, rice, tomato, cotton, fruitfly, mice, sheep, etc.) to suit human requirements.

Genetic engineering was started by **Paul Berg** (1972) who introduced gene of SV-40 (Semian virus 40) into a bacterium with the help of lambda (λ) phage. He is regarded as the **"Father of Genetic Engineering".** Berg was awarded the Nobel Prize in 1980.

Bacterial plasmid was discovered by **William Hays** and **Joshua Lederberg** in 1952. The DNA polymerase was investigated by **A. Kornberg** and coworkers in *E.coli* in 1956 and is now known as **DNA polymerase I** (DNA pol I). Restriction endonuclease was isolated for the first time by **Steward Linn** and **Warner Arber** in 1962 in bacterium *E.coli.*

The first recombinant DNA was constructed by **Stanley Cohen** and **Herbert Boyer** in 1972. The p B R 322 vector was the first artificial cloning vector constructed in 1977 by **Boliver** and **Rodriguez.**

Jeffey S.Chamberlain *et al.,* (1993) of Human Genome Project Center, Michigan University, U.S.A. have cured mice that inherited a neuromuscular disease. **Sir Alec Jeffreys** (1984) invented the DNA fingerprinting technique at Leicester University, United Kingdom. **Dr. V.K. Kashyap** and **Dr. Lal Ji Singh** of CCMB, Hyderabad, started the fingerprinting technology in India.

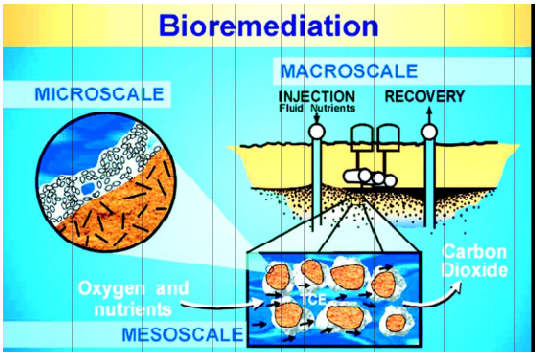
The sequencing of the entire human genome was accomplished by the Human Genome project (HPG) by 2003. It was a cooperative international effort and began in 1990 and involved hundreds of scientists (see **Becker** *et al.,* 2007).

Some Nicknames of Genetic Engineering

1. Molecular scissor – Restriction endonuclease
2. Chemical scalpel – Restriction endonuclease
3. Molecular sutures – Ligases
4. Molecular glue – T_4 ligase (It joins DNA fragments by forming **phosphodiester bonds**)
5. Workhouse for gene cloning – pBR322 (an artificial plasmid)
6. Gene taxi – Plasmid

CHAPTER

1



Basic Principles of Gene Cloning

Chapter Contents

1.1. *Biotechnology and Genetic Engineering*

1.2. *Genetic Engineering*

1.3. *What is Gene Cloning ?*

1.4. *Scope of Genetic Engineering*

1.1. BIOTECHNOLOGY AND GENETIC ENGINEERING

Biotechnology is an applied biological science. The term 'biotechnology' was coined by a Hungarian engineer, **Karl Ereky** (1917) to describe a process for large scale production of pigs using sugarbeet as food. **Biotechnology** can be defined as the use of biological organisms (such as viruses, prokaryotes and eukaryotes) or their components (such as systems and processes) to generate **products** and **services** useful to human beings. Examples of products are alcohol in the brewing industry and newer products such as human insulin from genetically engineered bacteria. Examples of services are treatment of sewage or detection of pollution using a biosensor. Here, the process rather than end-product is important.

Biotechnology does not include the use of complete animals and plants. This is mainly because exploitation of whole plants and animals is the subject of already well-established disciplines of agriculture, horticulture and animal husbandry.

Some Standard Definitions of Biotechnology

1. Biotechnology is ''the science of applied

biological process" (*Biotechnology*. A Dutch perspective, 1981).

2. Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service. (*The Organization for Economic Cooperation and Development* (*OECD*), *1981*).
3. Application of biological organisms, systems or processes to manufacturing and service industries (*British Biotechnologists*).
4. The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells and parts thereof (*European Federation of Biotechnology* (*FEB*), *1981*, O'Sullivan 1981).

Applications of biotechnology.

5. "A technology using biological phenomena for copying and manufacturing various kinds of useful substances" (*Japanese Biotechnologists*).
6. 'The application of biochemistry, biology, microbiology and chemical engineering to industrial processes and products and/or environment' (*International Union of Pure and Applied Chemistry* (*IUPAC*) 1981).
7. "The controlled use of biological agents, such as microorganisms or cellular components for beneficial use" (*US National Science Foundation*).

Lastly, in the definition given by OECD, "scientific and engineering principles" refer to microbiology, genetics, biochemistry, etc., and "biological agents" mean microorganisms, enzymes, plant and animal cells. The meaning of all these definitions of biotechnology which are given by different organizations of world are more or less similar.

1. Old Biotechnology

Human beings have been using microorganisms such as bacteria, yeasts and molds for thousands of years to obtain products such as curd, fermented drinks (wine), leavened bread, vinegar, etc. But they did not know that what microorganisms were involved in these processes? Microorganisms were first used to produce some organic compounds such as citric acid following the First World War. Subsequently, microorganisms were employed to generate a variety of products (*e.g.*, biofertilizers, bioinsecticides, vaccines, antibiotics, vitamins, food, single cell proteins). In addition, animal and plant, cell cultured *in vitro* are used to obtain several valuable products (*e.g.*, haploid plants, somatic hybrids, somaclonal variants, vaccines, antibodies and other animal proteins, pharmaceutical proteins. In all these processes, only the natural capabilities of the organisms and cells are exploited. As the understanding of microbes and molecular biology has grown, biotechnologists have been able to increase the output of traditional microorganism by creating an environment in which they multiply quickly and can be used for large-scale production. For example, **genetic improvement** have increased the levels of production. Thus, penicillin (an

Penicillin.

antibiotic) yield has been improved by a factor of about 1000. But the types of products obtained have remained the same, as those obtained from the natural strains/cell lines. All these activities come under old biotechnology.

2. New or Modern Biotechnology

In 1971, the technique of recombinant DNA technology was developed. **Genetic engineering** is a popular term for **recombinant DNA technology.** Recombinant DNA techniques permit the isolation of desired gene from any organism, and its transfer and expression into the organism of choice (Box 1.1).

Box 1.1.

The biotechnological application of genetic engineering requires *four* main stages :

1. Isolation of the required gene;
2. Inserting the gene into the bacteria;
3. Inducing the bacterial gene to start synthesizing the product;
4. Harvesting of that product.

Insulin.

A living organism that has been modified biogenetically by inserting DNA (gene) from another organism is called **transgenic organism.** Transgenic microorganisms are produced with a view to obtain novel pharmaceutical proteins. For example, human insulin is being commercially produced from a transgenic *Escherichia coli* strain that contains and expresses the human insulin gene. Proteins produced by **transgenes** (genes expressed in transgenic cells/organisms), are called **recombinant proteins.** Many valuable recombinant proteins are also being produced, using transgenic animal cell lines and transgenic plants (Table 1.1).

Table 1.1. Some important applications of transgenic organisms in agriculture, industry and human health.

Microorganism/Cell	Agriculture	Industry and human
1. Microorganism	–	Novel pharmaceutical proteins vaccines, new products.
2. Animal cells	–	New pharmaceutical proteins, vaccines, antibodies.
3. Plant cells	Transgenic crops [genetically modified (GM) crops] with resistance to herbicides, insects, viruses; modified quality; improved nutrition.	New pharmaceutical proteins; New high value products, genetically modified (GM) food

The recombinant proteins cannot be naturally produced by the concerned cells/organisms. These proteins are produced by the specific transgenes that were introduced into them. At the same time, a number of these proteins of great medicinal value could not be produced on a commercial scale using the non-transgenic cells or organisms. The production technologies based on genetic engineering are often referred to as **modern biotechnology.**

Transgenic plants.

1.2. GENETIC ENGINEERING

The recent off-shoot of biotechnology research is **genetic engineering** which involves gene splicing, recombinant DNA cloning and tissue culture technology. **Smith** (1996) has defined genetic engineering as "the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation in which they do not naturally occur but in which they continue propagation". The relationship of biotechnology and genetic engineering has been depicted in Fig. 1.1.

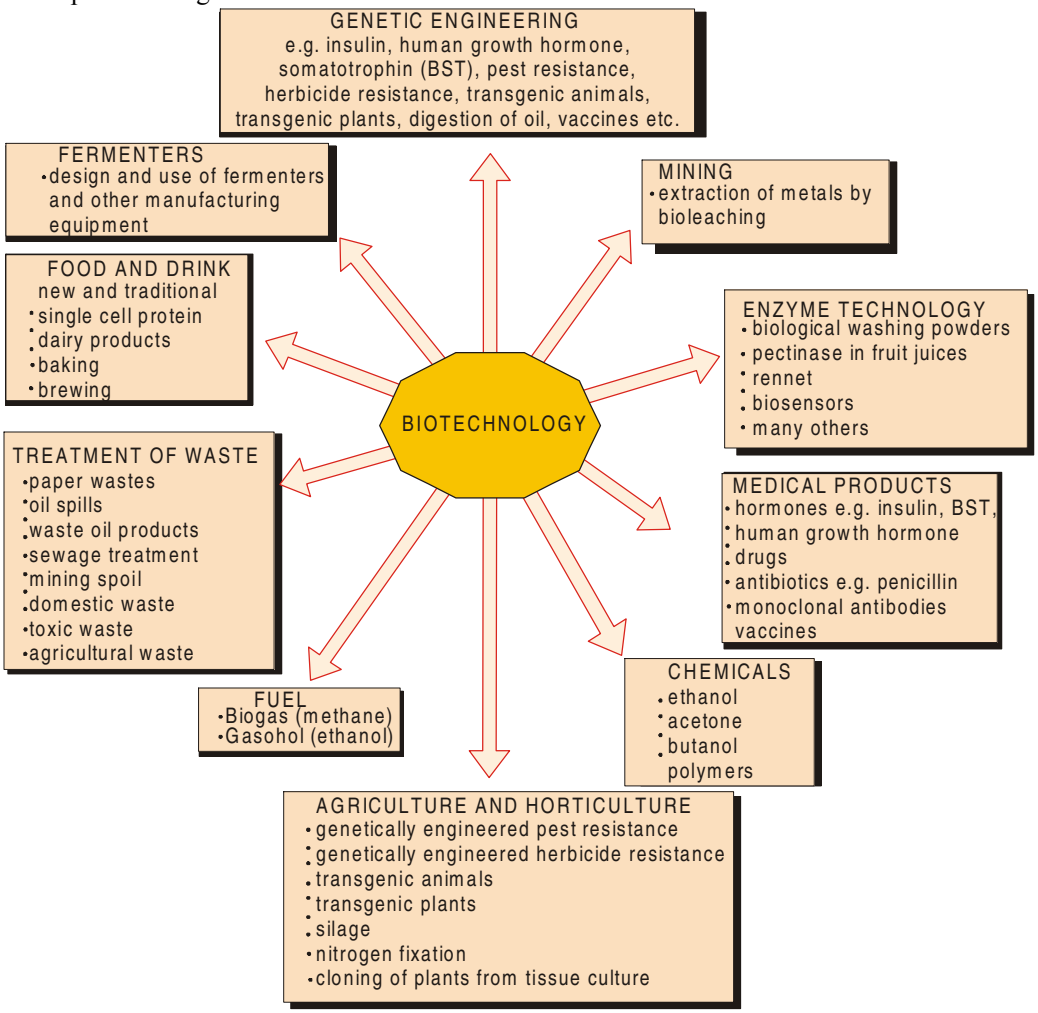


Fig. 1.1. Some of the applications of biotechnology. Categories are not rigid and may overlap. Genetic engineering is an important technique in biotechnology. It can be involved in any of the categories shown in order to improve microorganisms, plants and animals.

Protein Engineering

It is a new field of biotechnology in which mutagenesis techniques are used to develop new enzymes for biotechnological purposes. For example, careful alterations to the amino acid sequences of **subtilisin,** an enzyme used in biological washing powders have resulted in engineering versions with greater resistances to the thermal and bleaching (oxidative) stresses encountered in washing machines.

History of Genetic Engineering and Development of Transgenic Technology

1869 **Miescher** first isolated DNA from white blood cells harvested from pus-soaked bandages obtained from a nearby hospital.

1944 **Avery** provided evidence that DNA, rather protein, carries the genetic information during bacterial transformation.

1953 **Watson** and **Crick** proposed double-helix model for DNA structure based on X-ray results of **Franklin** and **Wilkins.**

1955 **Kornberg** discovered DNA polymerase, the enzyme now used to produce labeled DNA probes.

1961 **Marmur** and **Doty** discovered DNA renaturation establishing the specificity and feasibility of nucleic acid hybridization reactions.

1962 **Arber** provided the first evidence for the existence of DNA restriction nucleases, leading to their purification and use in DNA sequence characterization by **Nathans** and **H.Smith.**

1964 **Lederberg** and **Messelson** coined the term *restriction endonuclease* to describe the nuclease enzymes that destroys ('restrict') any foreign DNA entering the host cell.

1966 **Nirenberg, Ochoa** and **Khorana** explained the genetic code.

1967 **Gellert** discovered DNA ligase, the enzyme used to join DNA fragments together.

1968 **Messelson** and **Yuan** isolated and studied the first restriction endonuclease from *E.coli* K12.

1969 **Howard Temin** and **Davin Baltimore** independently discovered the enzyme reverse *transcriptase* from retroviruses. Later on, this was used to construct a DNA called complementary DNA (cDNA) from any RNA.

1972 **R.A.Dixon** and **J.R.Postgate** transferred *nif*-genes of *Klebsiella pneumoniae* (a non-symbiotic nitrogen fixing bacterium) into *E.coli* cells by conjugation.

1972-1973 DNA cloning or recombinant DNA tehcniques were developed by the laboratories of **Boyer, Cohen** and **Berg** and their colleagues at Stanford University and University of California at San Francisco.

1973 **S.Cohen** and **H.Boyer** developed a recombinant plasmid (pSC 101) which when used as vector replicated well in the bacterial host cell.

1975 **Edwin M.Southern** developed a technique (gel-transfer hybridization) for the detection of specific DNA fragments for isolation of a gene from complex mixture of DNA. This method is called *Southern blotting technique.*

1975-1977 **Sanger** and **Barrell** and **Maxam** and **Gilbert** developed rapid DNA-sequencing method.

1977 **K.Itakura, T.Hirose, R.Crea,** and colleagues got one of the first successes in the production of a recombinant protein, the hormone **somatostatin** by *E.coli.*

1981-1982 **Palmiter** and **Brinster** produced transgenic mice; **Sprealing** and **Rubin** produced transgenic fruit flies.

1981 **R.A.Hitzeman, F.E.Hagie, H.L.Levine** and collegues made the first use of yeast (*Saccharomyces cerevisiae*) in the production of recombinant protein, *i.e.*, interferon.

1982 **GenBank,** NIH's public genetic sequence database was established at Losa Alamos National Laboratory.

1983 **Gordon** and **Ruddle** first used the term transgenic in case of animals.

1984 **Alec Jeffreys** developed the technique of DNA fingerprinting. He demonstrated that human chromosomes had regions of non-coding DNA. These are called **mini-satellites** and can be used to identify individuals. This is called **DNA fingerprinting.**

1985 **Kary Mullis** invented PCR technique.

1987 **Capecchi** and **Smithies** introduced methods for performing targeted gene replacement in mouse embryonic stem cells.

1988 **D.W.Salter** and **L.B.Crittenden** have produced an ALV (Avian Leukosis Virus)-resistant strain of the chicken.

1989 **Fields** and **Song** developed the yeast two- hybrid system for identifying and studying protein interactions.

1989 **Olson** and colleagues described sequence- tagged sites, unique stretches of DNA that are used to make physical maps of human chromosomes.

1990 **A.J. Clark** produced a transgenic sheet that could secrete **factor IX** (a blood clotting protein) in the milk.

1990 **Lipman** and colleagues released BLAST (*i.e.*, Basic Local Alignment Search Tool), an aligorithm used to search for homology between DNA and protein sequences. (*Algorism* is any method of computation using Arabic notation).

1990 **Simon** and colleagues studied how to efficiently use bacterial artificial chromosome, BACs, to carry large pieces of cloned human DNA for sequencing.

1991 **Lalji Singh** at CCMB, Hyderabad developed a new technique of DNA fingerprinting by using BKM-DNA probe (BKM stands for banded krait minor satellite).

1991 **Hood** and **Hunkapillar** introduced new automated DNA sequence technology.

1995 **Venter** and colleagues sequenced the first complete genome of the bacterium *Haemophilus influenzae.*

1996 **Goffeau** and an international consortium of researchers announced the completion of the first genome sequence of an eukaryote, the yeast *Saccharomyces cerevisiae.*

1996 **Andrew Smith** and **Ute Kramer** at the University of Oxford have transferred the genes responsible for the super-accumulation of nickel from slow-growing *Alyssum lesbiacum* into fast-growing plant such as cauliflower.

1996-1997 **Lockhart** and colleagues and **Brown** and **DeRisi** produced DNA microarrays, which allows the simultaneous monitoring of thousands of genes.

1998 **Sulston** and **Waterston** and colleagues produced the first complete sequence of a multicellular organism, the nematode worm *Caenorhabditis elegans.*

2000 **J.Tal** used adeno-associated virus-based vectors in gene therapy.

2001 Consortia of researchers announce the completion of draft human genome sequence.

2007 **Richard Gross** used plant-oil to develop **'bio-plastic'** which was then broken down into fuel (bio diesel) with the help of a naturally occurring enzyme, **cutinase** of parasites which eat through shiny surfaces of tree leaves. The gene of this enzyme was cloned in bacterium *E.coli* for its mass production (see **M.Wald,** Indian Express April 10, 2007).

Some Milestones of Recombinant Technology

1976 First prenatal diagnosis was done by using gene specific probe.

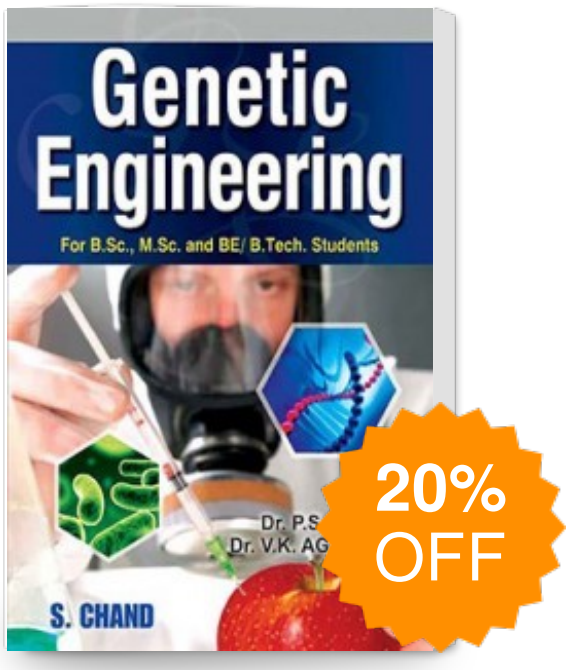
1977 Development of the technique for rapid DNA sequencing; discovery of split genes and somatostanin by recombinant DNA (rDNA).

1979 Insulin was synthesized by using rDNA; first viral antigen.

1980 Gene synthesis machines were developed. Total synthesis of IFN-A (human leukocyte interferon gene), 514 base pairs long, was achieved and published.

1981 Foot and mouth disease viral antigen was cloned.

Genetic Engineering



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