Biotechnology : Scope and Importance

A. WHAT IS BIOTECHNOLOGY ?

The term biotechnology was coined in 1917 by a Hungarian Engineer, **Karl Ereky**, to describe a process for large scale production of pigs. According to him all types of work are biotechnology by which products are produced from raw materials using living organisms. During the end of 20th century biotechnology emerged as a new discipline of biology integrating with technology; but the route of biotechnology lies in biology. There was no sudden sprout of this discipline, but some of the methods for production of products were developed centuries back. Therefore, biotechnology is concerned with exploitation of biological components for production of useful products. Biotechnology is defined by different organisations in different ways. It has been broadly defined as, "the development and utilization of biological processes, forms and systems for obtaining maximum benefits to man and other forms of life". Biotechnology is "the science of applied biological process" (*Biotechnology* : A Dutch Perspective, 1981). Following are some of the definitions given by other organisations :

These scientists are developing new vaccines against the HIV viruses.

- *Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service* [The Organisation for Economic Cooperation and Development (OECD), 1981].
- *The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological application of the capabilities of microorganisms, cultured tissue, cells, and parts their of* [The European Federation of Biotechnology (EFB), 1981; O'Sullivan, 1981].
- *The application of biochemistry, biology, microbiology and chemical engineering to industrial process and products and on environment* [International Union of Pure and Applied Chemistry (IUPAC), 1981.]
- Biotechnology is the "*controlled use of biological agents such as microorganisms or cellular components for beneficial use*" (U.S. National Science Federation).

In the definition given by OECD, "scientific and engineering principles" refer to microbiology, genetics, biochemistry, etc. and "biological agents" means microorganisms, enzymes, plant and animal cells. The meaning of these three definitions and others given by many organisations are more or less similar.

A unified definition of genetic engineering has been given by Smith (1996) 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 into a host organism in which they do not naturally occur but in which they are capable of continued propagation*".

B. HISTORY OF BIOTECHNOLOGY

If we trace the origin of biotechnology, it is as old as human civilization. Development of biotechnology can be studied considering its growth that occurred in two phases: (*i*) the traditional (old) biotechnology, and (*ii*) the new (modern) biotechnology.

1. Traditional Biotechnology

The traditional biotechnology is really the kitchen technology developed by our ancestors using the fermenting bacteria. Kitchen technology is as old as human civilisation. During *vedic* period (5000–7000 BC), *Aryans* had been performing daily *Agnihotra* or *Yajna*. One of the materials used in Yajna is animal fat (i.e. *ghee*) which is a fermented product of milk. Similarly, the divine '*soma*' (a fermented microbial product used as beverage) had been offered to God. Summarians and Babylonians (6000 BC) were drinking the beer. Egyptians were baking leavened bread by 4000 BC. Preparation of curd, *ghee*, wine beer, vinegar, etc. was the kitchen technology. In spite of all development, preparation of curd, *ghee*, vinegar, alcoholic beverages, jalebi, idli, dosa, have become an art of the kitchen of all Indians (Table 1.1).

Brewing is one of the ancient methods of fermentation.

new small molecules as potential drug candidates. Therefore, interaction of some molecules can be studied in greater detail.

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 cure so far. For example, a specific gene (*Her-2 Neu*) over-expresses in breast cancer patients. A designed drug (Herceptin) is good for treatment of breast cancer. Thus, the field of genomics has helped the growth of pharmacologic, toxicologic and protein studies on animals, therefore, the new areas are called pharmacogenomics, toxicogenomics and proteogenomics, respectively (*see* Chapter 8).

(vi) Bioinformatics: It is a new field of biotechnology linked with information technology. Bioinformatics may be defined *as application of information sciences (mathematics, statistics, and computer sciences) to increase the understanding of biology, biochemistry and biological data.* The most remarkable success of bioinformatics to date has been its use in the 'shotgun sequencing' (breaking of a large piece of DNA into smaller fragments) of human genome.

D. BIOTECHNOLOGY IN INDIA AND GLOBAL TRENDS

1. Biotechnology in India

In most of the developing countries, the recombinant DNA technology has become the major thrusts. In 1982, Government of India set up an official agency, 'the National Biotechnology Board' (NBTB) which started functioning under the Department of Science and Technology (DST). In 1986, NBTB was replaced with a full-fledged department, the Department of Biotechnology (DBT), under the Ministry of Science and Technology for planning, promotion and coordination of various biotechnological programmes.

The DBT is making effort in promoting post graduate education and research. Special M.Sc. courses in Biotechnology in selected group of institution with scholarship are provided by the DBT. The selection of students is done via National Test. In addition, it also provides trained manpower for the rapidly growing biotech industry. It has also raised the level of biology education in certain areas of biotechnology in the country. Moreover, a considerable amount of basic biochemical and molecular biology is imported in these courses.

Today, India has the DBT, DST, CSIR, ICAR, ICMR and IARI, and other agencies which are working under the Government. These agencies and the other National and International Industries are manufacturing Biotech products and marketing them after clinical trials. A Technology Development Board (TDB) has been set up by the Government for the promotion of product development. The TDB works with universities, industries and the National Institutes. The Technology Information, Forecasting and Assessment Council (TIFAC) has prepared a 'Vision 2020' document which consists of biotechnology also.

There are over 30 companies in India which are producing the modern biotech products such as Wipro, Reliance, Life Science, Pennetia Biotech Ltd.

Since 1980s, India has supported a lot to biotechnology industry and its products. Teaching and research of biotechnology have been included in University's syllabi both at Under Graduate and Post graduate levels. DBT- supported departments are running in several Universities and Institutions. It is hoped that India will play a key role in future as one of the largest market of the world, and as a producer of biotech products.

(i) **International Centre for Genetic Engineering and Biotechnology (ICGEB).** The United Nations Industrial Development Organisation (UNIDO) recognised the potential of genetic engineering and biotechnology for promoting the economic progress of the developing countries.

The initiation taken by UNIDO has led to the foundation of ICGEB. In 1981, in a meeting convened by UNIDO it was proposed to establish an international centre of excellence to foster biotechnology in the developing world. In 1982, this concept was approved by a high level conference of developed and developing nations in Belgrade. The statutes of the centre were signed by 26 countries with the entry into force of statutes on February 3, 1994. The ICGEB has become a fully autonomous international organisation composing of at present 33 member states.

The ICGEB has its two centres, one located in Trieste (Italy), and the other in Jawaharlal Nehru University, New Delhi (India). The Trieste component is currently occupying about 5,700 m^2 area, whereas the New Delhi component is occupying about 10,000 m^2 area. This centre is functioning in a proper way since 1982.

The organs of ICGEB are the Secretariat, the Board of Governors and the Council of Scientific Advisors. The secretariat component is the Director, two Heads of the components and the scientific and administrative staff operating with the framework of the ICGEB programme. The Board of Governors consists of a representative of each Member State. The Council of Scientific Advisors is composed of eminent scientists and overseas scientific excellence of ICGEB. Funds are provided by the government of Italy and India. From 1999, all Member States have started to finance ICGEB through a scale of assessment adopted by the Board of Governors.

The activities of ICGEB are aimed specifically at strengthening the R & D capability of its Member States by :

(*i*) providing the developing countries with a necessary 'critical mass' environment to pursue and advance the research in biotechnology; host research facilities that are technology and capital demanding and, therefore, inaccessible to the great majority of developing countries,

(*ii*) training schemes and collaborative research with affiliated centres to ensure that significant members of scientists from Member states are trained in state-of-art technology, in areas of direct relevance to the specific problems of their countries,

(*iii*) acting as the coordinating hub of network of affliated centres that serve as localized nodes for distribution of information and resources located at ICGEB.

Initially a total of six centres were set up at various Universities/Institution namely, Jawaharlal Nehru University (New Delhi), Madurai Kamraj university (Madurai), Tamil Nadu Agriculture University (Coimbatore), National Botanical Institute (Lucknow), and Bose Institute (Kolkata). In 1997, a seventh centre was established at the University of Delhi (South Campus).

In addition, the other centres for biotechnology in India are : Indian Agricultural Research Institute (IARI), Jawaharlal Nehru University (JNU), Delhi University, Indian Veterinary Research Institute (IVRI), Izzatnagar (U.P.); Central Food and Technology Research Institute (CFTRI), Mysore; National Dairy Research Institute (NDRI), Karnal (Haryana); Malaria Research Centre (MRC), Delhi; Regional Research Laboratory (RRL) Jammu; Central Drug Research Institute (CDRI) and Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow; Indian Institute of Technology (IIT), Kanpur, Madras, Bombay and New Delhi. Other centres to which DBT has provided infrastructural facilities are Banaras Hindu University, Varanasi; Allahabad University; M.K. University, Madurai; Anna University (Madras); Indian Institute of Science (Bangalore); Pune University, Pune; All India Institute of Medical Sciences, New Delhi; Bhabha Atomic Research Centre (BARC), Bombay, etc. Facilities supported by DST are given in Table 1.6. Mr. Rajiv Gandhi, the Late Prime Minister of India, laid a foundation stone on October 4, 1988 at the Centre I.A.R.I. with the name "Lal Bahadur Centre for Biotechnology".

> Table 1.6. Infrastructural facilities supported by DBT, Government of India.

Facility	*Government Agency*
Blue green algae collection	Indian Agricultural Research Institute (I.A.R.I), New Delhi.
Plant tissue culture repository	National Bureau of Plant Genetic Resources (N.B.P.G.R.), New Delhi.
Microbial type cultural collection	Institute of Microbial Technology (I.M.Tech), Chandigarh
Animal tissue and culture collection	Pune University, Pune
Animal House	(*a*) Central Drug Research Institute (C.D.R.I.), Lucknow (*b*) Indian Institute of Sciences (I.I. Sc.), Bangalore (*c*) National Institute of Nutrition (N.I.N.), Hyderabad
Oligonucleotide synthesis	I.I.Sc., Bangalore
Biochemical Engineering and Pilot Plant	I.M.T. Chandigarh
Biotechnology Information Centre (BIC)	(*a*) Genetic Engineering (M.K. University, Madurai; Bose Institute, Kolkata; Jawaharlal Nehru University, New Delhi) (*b*) Animal Cell Culture and Virology (Pune University, Pune) (*c*) Oncogene Research (Central Cellular and Molecular Biology, Hyderabad) (*d*) Immunology (National Institute of Immunology, New Delhi) (*e*) Enzyme Engineering, Immobilised Biocatalysts, Microbial Fermentation and Bioprocess Engineering (I.M.Tech, Chandigarh)

Many public and private institutions working under the Government departments and organisations have advised the DBT to formulate the biotechnology programmes under the following areas : (*i*) Plant molecular biology and agricultural biotechnology, (*ii*) Biochemical engineering, process optimisation and bioconversion, (*iii*) Aquaculture and marine biotechnology, (*iv*) Fuel, fodder, biomass and green cover, (*v*) Medical biotechnology, (*vi*) Microbial and industrial biotechnology, (*vii*) Large scale use of biotechnology, (*viii*) Integrated systems in biotechnology, (*ix*) Veterinary biotechnology and (*x*) Infrastructural facilities.

A domestic biogas digester in an Indian village, using animal manure and producing methane for cooking and heating water.

Pharmaceutical industry in India is very strong and vibrant with expertise for chemical drugs. It has little experience in biotech diagnostics and no experience in biotech therapeutics. Moreover, pharma industry is located between Mumbai and Ahmedabad (90% of drug production in India is in Gujarat and Maharashtra). There is no Government institution or university with expertise in this area to help pharma

industry. However, for a variety of reasons, the Indian pharmaceutical industry will sooner or later enter in manufacturing of biotechnology based diagnostics and therapeutics (Padh, 1996).

(ii) Needs for Future Development. A few developing countries like India, have scientists and technologists related to biotechnology where national strategies of development in biotechnology could be implemented. The scientific and technical manpower has to be properly shifted towards new biotechnology with the aim to produce expertise in biotechnology. In a keynote address, Bachhawat and Banerjee (1985) have described the impact of biotechnology on third world countries. They emphasized "Indian bioscientist must be trained to utilize their knowledge and expertise for application and orientation, for example, a microbiologist must be trained in microbial genetics to be really useful in fermentation technology, or a botanist must be trained in cell culture, protoplast fusion or DNA recombination for practical utility and similarly, people from traditional disciplines in life science may be trained to reorient their knowledge towards application and process of training readjusted according to need."

It is, therefore, necessary to encourage the biotechnological programmes at industrial and educational levels. In higher education, teaching of biotechnology should be compulsory for undergraduate students to expand their understanding and knowledge of the scientific and engineering principles underlying biotechnology. Countries like U.S.A., France, Germany and Japan have taken this issue seriously.

In India, most of the universities have started teaching biotechonology at under-graduate level. However, at post graduate level teaching and research have been initiated only by a few universities/ institutes on all India entrance test basis. Government of India has selected many thrust areas of national and international relevance as described earlier.

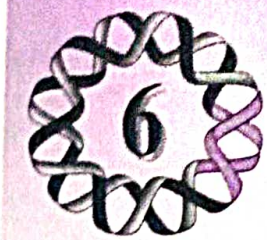
2. Global Scenario

Biotechnology products are increasing in world market day-by-day. The high value added biotechnology product to be used in medical field are now in domination for the last few years. The countries which have boosted up biotechnology R & D during the past two decades are the U.S.A., U.K., Japan, France, Australia, Russia, Poland, Germany, as well as India (among the developing countries). The most effective means of promoting international cooperation is through networks. The international networks devoted to applied microbiology/biotechnology are the Regional Microbiology Network for South-East Asia, and the network of Microbiological Resource Centres (MICRENs).

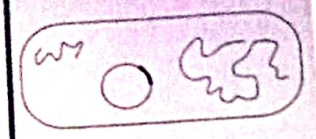
To foster biotechnology inventions, the U.S.A. promotes enterprises through policy development and support. Funding for basic scientific research at the National Institute of Health (NIH) has been supported. The U.S. administration has boosted up the process for improving new medicines so that these may be quickly and safely available in market. The private sector research investment and small business development have also been encouraged through the incentives. Position of therapeutic proteins and vaccines up to June 1998 by the USA has been given in Table 1.7.

> Table 1.7. Position of therapeutic proteins and vaccines up to June 1998 (based on Pharmaceutical Research and Manufacturers of America; Biotechnology Industry Organisation).

Therapeutic proteins/vaccines	*Status*	
	Approved	*In development*
Blood clotting factor	3	3
Gene therapy	0	38
Growth factors	21	8



Introduction to Genetic Engineering



Genetic engineering primarily involves the ***manipulation of genetic material (DNA)*** to achieve the desired goal in a pre-determined way. Some ***other terms*** are also in common use to describe genetic engineering.

- ***Gene manipulation***
- ***Recombinant DNA (rDNA) technology***
- ***Gene cloning (molecular cloning)***
- ***Genetic modifications***
- ***New genetics.***

BRIEF HISTORY OF RECOMBINANT DNA TECHNOLOGY

The present day DNA technology has its roots in the experiments performed by ***Boyer and Cohen in 1973.*** In their experiments, they successfully recombined two plasmids (pSC 101 and pSC 102) and cloned the new plasmid in *E.coli*. Plasmid pSC 101 possesses a gene resistant to antibiotic tetracycline while plasmid pSC 102 contains a gene resistant to another antibiotic kanamycin. The newly developed recombined plasmid when incorporated into the bacteria exhibited resistance to both the antibiotics-tetracycline and kanamycin.

The second set of experiments of Boyer and Cohen were more organized. A gene encoding a protein (required to form rRNA) was isolated from the cells of African clawed frog *Xenophs laevis*, by use of a restriction endonuclease enzyme (*ECoRI*). The same enzyme was used to cut open plasmid pSC 101 DNA. Frog DNA fragments and plasmid DNA fragments were mixed, and pairing occurred between the complementary base pairs. By the addition of the enzyme DNA ligase, a recombined plasmid DNA was developed. These new plasmids, when introduced into *E.coli*, and grown on a nutrient medium resulted in the production of an extra protein (i.e. the frog protein). Thus, the genes of a frog could be successfully transplanted, and expressed in *E.coli*. This made the real beginning of modern rDNA technology and laid foundations for the present day molecular biotechnology.

Some biotechnologists who admire Boyer-Cohen experiments divide the subject into two chronological categories.

1. ***BBC***-biotechnology ***B***efore ***B***oyer and ***C***ohen.
2. ***ABC***-biotechnology ***A***fter ***B***oyer and ***C***ohen.

More information on the historical developments of genetic engineering and biotechnology is given under the scope of biotechnology (Chapter 1).

An outline of recombinant DNA technology

There are many diverse and complex techniques involved in gene manipulation. However, the basic principles of recombinant DNA technology are reasonably simple, and broadly involve the following stages (***Fig.* 6.1**).

1. Generation of DNA fragments and selection of the desired piece of DNA (e.g. a human gene).

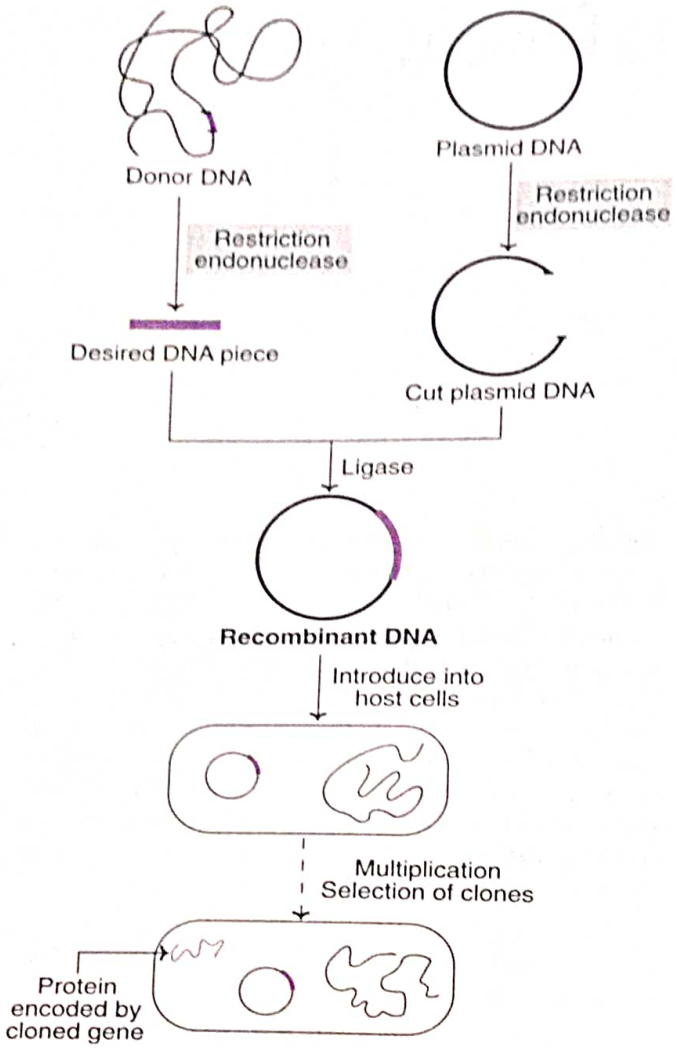


Fig. 6.1 : The basic principle of recombinant DNA technology.

2. Insertion of the selected DNA into a cloning vector (e.g. a plasmid) to create a ***recombinant DNA*** or ***chimeric DNA*** (Chimera is a monster in Greek mythology that has a lion's head, a goat's body and a serpent's tail. This may be comparable to Narasimha in Indian mythology).

3. Introduction of the recombinant vectors into host cells (e.g. bacteria).

4. Multiplication and selection of clones containing the recombinant molecules.

5. Expression of the gene to produce the desired product.

Recombinant DNA technology with special reference to the following aspects is described in this chapter.

1. Molecular tools of genetic engineering.
2. Host cells-the factories of cloning.
3. Vectors-the cloning vehicles.
4. Methods of gene transfer.
5. Gene cloning strategies.
6. Genetic engineering guidelines.
7. The future of genetic engineering.

MOLECULAR TOOLS OF GENETIC ENGINEERING

An engineer is a person who designs, constructs (e.g. bridges, canals, railways) and manipulates according to a set plan. The term genetic engineer may be appropriate for an individual who is involved in genetic manipulations. The ***genetic engineer's toolkit*** or molecular tools namely the enzymes most commonly used in recombinant DNA experiments are briefly described.

RESTRICTION ENDONUCLEASES— DNA CUTTING ENZYMES

Restriction endonucleases are one of the most important groups of enzymes for the manipulation of DNA. These are the bacterial enzymes that can cut/split DNA (from any source) at specific sites. They were first discovered in *E.coli* restricting the replication of bacteriophages, by cutting the viral DNA (The host *E.coli* DNA is protected from cleavage by addition of methyl groups). Thus, the enzymes that restrict the viral replication are known as ***restriction enzymes*** or restriction endonucleases.

Hundreds of restriction endonucleases have been isolated from bacteria, and some of them are commercially available. The progress and growth of biotechnology is unimaginable without the availability of restriction enzymes.

Nomenclature

Restriction endonucleases are named by a standard procedure, with particular reference to the bacteria from which they are isolated. The first letter (in italics) of the enzymes indicates the genus

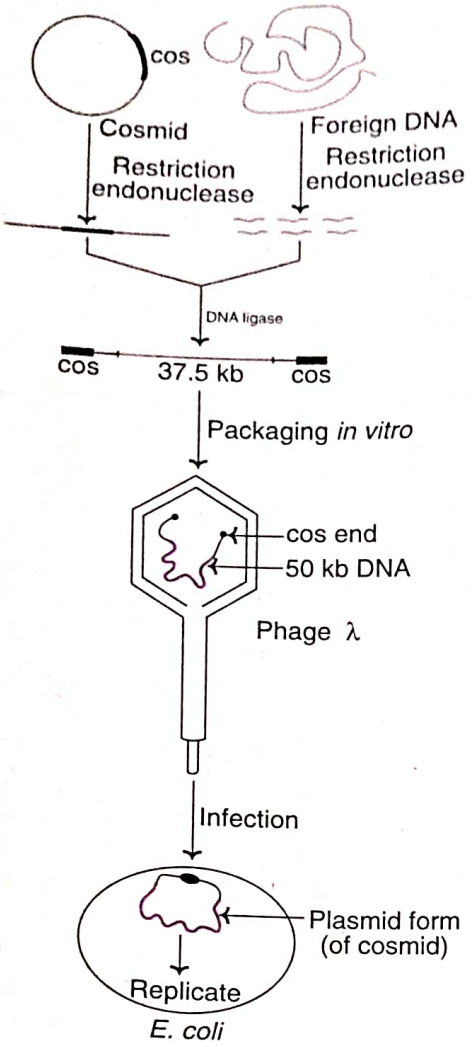


Fig. 6.10 : *Cosmids as vectors.*

ARTIFICIAL CHROMOSOME VECTORS

Human artificial chromosome (HAC)

Developed in 1997 (by H. Willard), human artificial chromosome is a ***synthetically produced vector DNA, possessing the characteristics of human chromosome***. HAC may be considered as a self-replicating microchromosome with a size ranging from 1/10th to 1/5th of a human chromosome. The advantage with HAC is that it can carry human genes that are too long. Further, HAC can carry genes to be introduced into the cells in gene therapy.

Yeast artificial chromosomes (YACs)

Introduced in 1987 (by M. Olson), yeast artificial chromosome (YAC) is a synthetic DNA that can accept large fragments of foreign DNA (particularly human DNA). It is thus possible to clone large DNA pieces by using YAC. YACs are the most sophisticated yeast vectors, and represent the ***largest capacity vectors*** available. They possess centromeric and telomeric regions, and therefore the recombinant DNA can be maintained like a yeast chromosome.

Bacterial artificial chromosomes (BACs)

The construction of BACs is based on one F-plasmid which is larger than the other plasmids used as cloning vectors. BACs can accept DNA inserts of around 300 kb. The advantage with bacterial artificial chromosome is that the instability problems of YACs can be avoided. In fact, a major part of the ***sequencing of human genome*** has been accomplished by using a library of BAC recombinant.

SHUTTLE VECTORS

The plasmid vectors that are specifically designed to ***replicate in two different hosts*** (say in *E.coli* and *Streptomyces* sp) are referred to as shuttle vectors. The origins of replication for two hosts are combined in one plasmid. Therefore, any foreign DNA fragment introduced into the vector can be expressed in either host. Further, shuttle vectors can be grown in one host and then shifted to another host (hence the name shuttle). A good number of eukaryotic vectors are shuttle vectors.

CHOICE OF A VECTOR

Among the several factors, the size of the foreign DNA is very important in the choice of vectors. The efficiency of this process in often crucial for determining the success of cloning. The size of DNA insert that can be accepted by different vectors is shown in ***Table 6.5***.

METHODS OF GENE TRANSFER

Introducing a foreign DNA (i.e. the gene) into the cells is an important task in biotechnology. The efficiency of this process is often crucial for determining the success of cloning. The most commonly employed gene transfer methods, namely transformation, conjugation, electroporation and lipofection, and direct transfer of DNA are briefly described.

Table 6.5 The different cloning vectors with the corresponding hosts and the sizes of foreign insert DNAs

Vector	*Host*	*Foreign insert DNA size*
Phage λ	*E. coli*	5–25 kb
Cosmid λ	*E. coli*	35–45 kb
Plasmid artifical chromosome (PAC)	*E. coli*	100–300 kb
Bacterial artificial chromosome (BAC)	*E. coli*	100–300 kb
Yeast chromosome	*S. cerevisiae*	200–2000 kb

TRANSFORMATION

Transformation is the method of introducing foreign DNA into bacterial cells (e.g. *E.coli*). The uptake of plasmid DNA by *E.coli* is carried out in ice-cold $CaCl_2$ (0-5°C), and a subsequent heat shock (37–45°C for about 90 sec). By this technique, the ***transformation frequency***, which refers to ***the fraction of cell population that can be transferred***, is reasonably good e.g. approximately one cell for 1000 (10^{-3}) cells.

Transformation efficiency : It refers to the number of transformants per microgram of added DNA. For *E.coli*, transformation by plasmid, the transformation efficiency is about 10^7 to 10^8 cells per microgram of intact plasmid DNA. The bacterial cells that can take up DNA are considered as competent. The competence can be enhanced by altering growth conditions.

The mechanism of the transformation process is not fully understood. It is believed that the $CaCl_2$ affects the cell wall, breaks at localized regions, and is also responsible for binding of DNA to cell surface. A brief heat shock (i.e. the sudden increase in temperature from 5°C to 40°C) stimulates DNA uptake. In general, large-sized DNAs are less efficient in transforming.

Other chemical methods for transformation

Calcium phosphate (in place of $CaCl_2$) is preferred for the transfer of DNA into cultured cells. Sometimes, calcium phosphate may result in precipitate and toxicity to the cells. Some workers use diethyl aminoethyl dextran (DEAE -dextran) for DNA transfer.

CONJUCTION

Conjugation is ***a natural microbial recombination process***. During conjugation, two live bacteria (a donor and a recipient) come together, join by cytoplasmic bridges and transfer single-stranded DNA (from donor to recipient). Inside the recipient cell, the new DNA may integrate with the chromosome (rather rare) or may remain free (as is the case with plasmids).

Conjugation can occur among the cells from different genera of bacteria (e.g *Salmonella* and *Shigella* cells). This is in contrast to transformation which takes place among the cells of a bacterial genus. Thus by conjugation, transfer of genes from two different and unrelated bacteria is possible.

The natural phenomenon of conjugation is exploited for gene transfer. This is achieved by transferring plasmid-insert DNA from one cell to another. In general, the plasmids lack conjugative functions and therefore, they are not as such capable of transferring DNA to the recipient cells. However, some plasmids with conjugative properties can be prepared and used.

ELECTROPORATION

Electroporation is based on the principle that high voltage electric pulses can induce cell plasma membranes to fuse. Thus, electroporation is a technique involving ***electric field-mediated membrane permeabilization.*** Electric shocks can also induce cellular uptake of exogenous DNA (believed to be via the pores formed by electric pulses) from the suspending solution. Electroporation is a simple and rapid technique for introducing genes into the cells from various organisms (microorganisms, plants and animals).

The basic technique of electroporation for transferring genes into mammalian cells is depicted in ***Fig. 6.11***. The cells are placed in a solution containing DNA and subjected to electrical shocks to cause holes in the membranes. The foreign DNA fragments enter through the holes into the cytoplasm and then to nucleus.

Electroporation is an effective way to transform *E.coli* cells containing plasmids with insert DNAs longer than 100 kb. The transformation efficiency is around 10^9 transformants per microgram of DNA

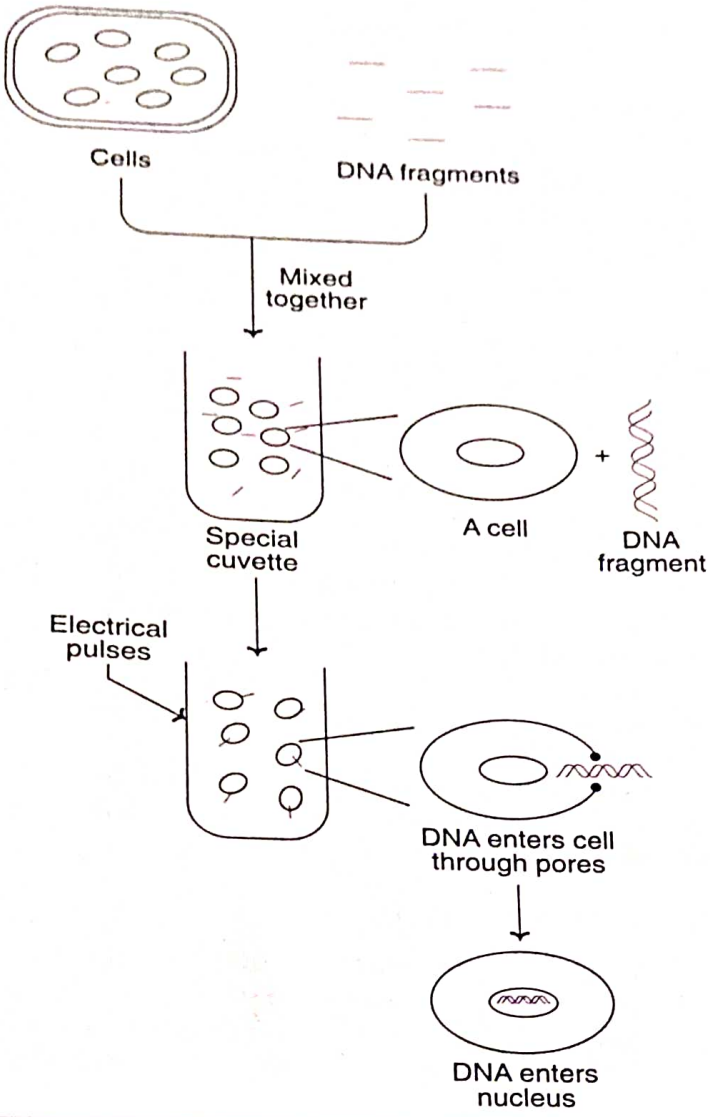


Fig. 6.11 : *Gene transfer by electroporation.*
(Note : *Magnification depicted on right side)*

for small plasmids (about 3kb) and about 10^6 for large plasmids (about 130 kb).

LIPOSOME-MEDIATED GENE TRANSFER

Liposomes are circular lipid molecules, which have an aqueous interior that can carry nucleic acids. Several techniques have been developed to encapsulate DNA in liposomes. The liposome-mediated gene transfer, referred to as ***lipofection***, is depicted in ***Fig. 6.12***.

On treatment of DNA fragment with liposomes, the DNA pieces get encapsulated inside liposomes. These liposomes can adher to cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus. The positively charged liposomes very efficiently complex with DNA, bind to cells and transfer DNA rapidly.

Lipofection is a very efficient technique and is used for the transfer of genes to bacterial, animal and plant cells.

The different methods of gene transfer are briefly described above. More details are given in the respective chapters applying these techniques to achieve gene transfer.

TRANSDUCTION

Sometimes, the foreign DNA can be packed inside animal viruses. These viruses can naturally

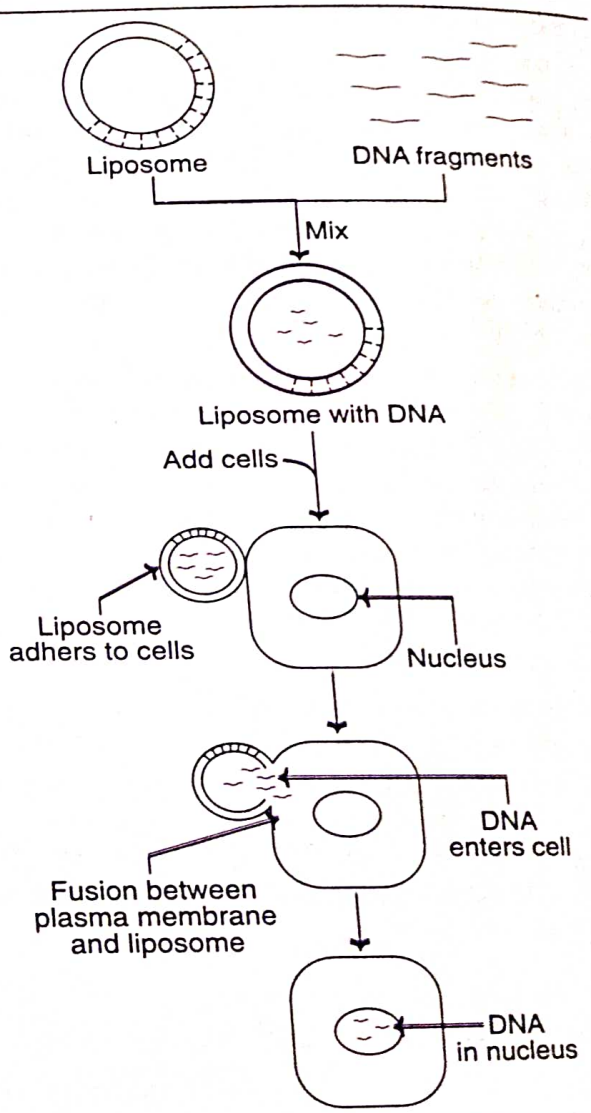


Fig. 6.12 : *Liposome-mediated gene transfer*
(Note : *For clarity, the native cell DNA is not show*

infect the cells and introduce the DNA into host cells. The transfer of DNA by this approach is referred to as transduction.

DIRECT TRANSFER OF DNA

It is possible to directly transfer the DNA into the cell nucleus. Microinjection and particle bombardment are the two techniques commonly used for this purpose.

Microinjection

DNA transfer by microinjection is generally used for the cultured cells. This technique is also useful to introduce DNA into large cells such as oocytes, eggs and the cells of early embryos.

Some more details on gene transfer methods including particle bombardment are given in Chapter 49.

The term ***transfection is used for the transfer DNA into eukaryotic cells,*** by various physical or chemical means.

GENE CLONING STRATEGIES

A clone refers to ***a group of*** organisms, cells, ***molecules*** or other objects, ***arising from a single individual.*** Clone and colony are almost synonymous.

Gene cloning strategies in relation to recombinant DNA technology broadly involve the following aspects (***Fig. 6.13***).

- Generation of desired DNA fragments.
- Insertion of these fragments into a cloning vector.
- Introduction of the vectors into host cells.
- Selection or screening of the recipient cells for the recombinant DNA molecules.

It is obvious that for a good understanding (or account) of gene cloning strategies, this chapter has to be learnt in detail. In addition, the reader must invariably refer gene libraries (Chapter 9) for ***cloning of genes and screening strategies,*** and the ***polymerase chain reaction*** (Chapter 8) for *in vitro* generation of large quantities of DNA. Further, gene cloning also involves the expression of genes which is described under ***manipulation of gene expression in host cells*** (Chapter 11).

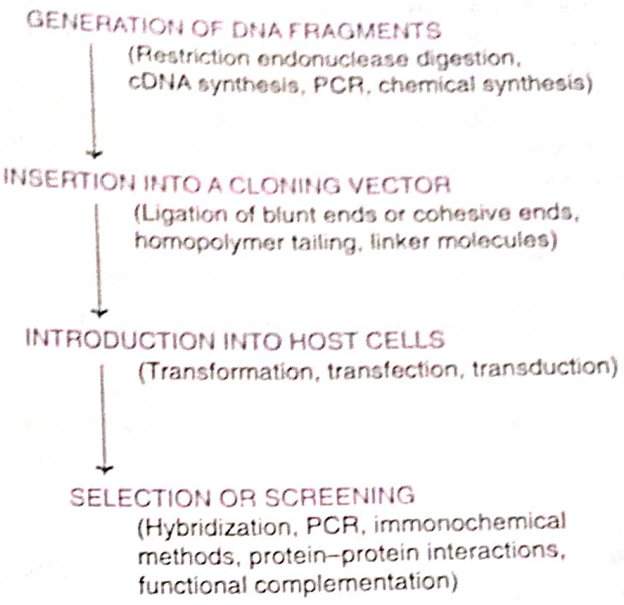


Fig. 6.13 : An overview of cloning strategies in recombinant DNA technology.

CLONING FROM GENOMIC DNA OR MRNA?

DNA represents the complete genetic material of an organism which is referred to as genome. Theoretically speaking, cloning from genomic DNA is supposed to be ideal. But the DNA contains non-coding sequences (introns), control regions and repetitive sequences. This complicates the cloning strategies, hence DNA as a source material is not preferred, by many workers. However, if the objective of cloning is to elucidate the control of gene expression, then genomic DNA has to be invariably used in cloning.

The use of mRNA in cloning is preferred for the following reasons.

- mRNA represents the actual genetic information being expressed.
- Selection and isolation mRNA is easy.
- As introns are removed during processing, mRNA reflects the coding sequence of the gene.
- The synthesis of recombinant protein is easy with mRNA cloning.

Besides the direct use of genomic DNA or mRNA, it is possible to synthesize DNA in the laboratory (Chapter 7), and use it in cloning

experiments. This approach is useful if the gene sequence is short and the complete sequence of amino acids is known.

The different strategies for the cloning of genomic DNA and mRNA are described under gene libraries (Chapter 9).

GENETIC ENGINEERING OF PLANTS

For additional information on the genetic engineering of plants, the reader must refer Chapter 49. Some details on the following aspects are given in that chapter

- Gene transfer methods (vector-mediated gene transfer — T DNA, plant viruses; direct DNA transfer — physical and chemical methods).
- Marker genes for plant transformation.
- Promoters and terminators.
- Transgene stability, expression and gene silencing.
- Chloroplast transformation.

GENETIC ENGINEERING GUIDELINES

With the success of Boyer-Cohen experiments (in 1973), it was realised that recombinant DNA technology could be used to create organisms with novel genes. This created worldwide commotion (among scientists, public and government officials) about the safety, ethics and unforeseen consequences of genetic manipulations. Some of the phrases quoted in media in those days are given.

- Manipulation of life.
- Playing God.
- Man made evolution.
- The most threatening scientific research.

It was feared that some new organisms, created inadvertently or deliberately for warfare, would cause epidemics and environmental catastrophes. Due to the fears of the dangerous consequences, a cautious approach on recombinant DNA experiments was suggested.

In 1974, a group of ten scientists led by Paul Berg wrote a letter that simultaneously appeared in the prestigeous journals-Nature, Science and Proceedings of the National Academy of Sciences. The dangers of DNA technology were printed out in that letter (highlights given below) :

"Recent advances in techniques for isolation and rejoining of segments of DNA now permit construction of biologically active recombinant DNA molecules in vitro. Although such experiments are likely to facilitate the solution of important theoretical and practical biological problems, they would also result in creation of novel types of DNA elements whose biological properties cannot be completely predicted. There is a serious concern that some of these DNA molecules could prove biologically hazardous".

The letter also appealed to molecular biologists worldwide for a moratorium on many kinds of recombinant DNA research, particularly those involving pathogenic organisms.

ASILOMAR RECOMMENDATIONS

In February 1975, a group of 139 scientists from 17 countries held a conference at Asilomar, a conference center in California, USA. They assured the uneasy public that the microorganisms used in DNA experiments were specifically bred and could not survive outside the laboratory. These scientists ***formulated guidelines and recommendations for conducting experiments in genetic engineering.***

NIH GUIDELINES

National Institute of Health (NIH), USA constituted the ***Recombinant DNA Advisory Committee (RAC)*** which issued a set of stringent guidelines to conduct research on DNA. RAC was in fact overseeing the research projects involving gene splicing and recombinant DNA.

Some of the important original NIH recommendations on recombinant DNA research relate to the following aspects.

- Physical (laboratory) containment levels for conducting expermiments.
- Biological containment-the host into which foreign DNA is inserted should not proliferate outside the laboratory or transfer its DNA into other organisms.

- For research on pathogenic organisms, elaborate, controlled and self-contained rooms were recommended.
- For research on less dangerous organisms, units equipped with high quality filter systems should be used.
- No deliberate release of any organism containing recombinant DNA into the environment.

It may be noted here that although the NIH guidelines did not have the legal status, most institutions, companies and scientists voluntarily complied.

Relaxation of NIH guidelines

It was in 1980, the original NIH guidelines were considerably relaxed by NIH-RAC, based on the experience and experimental data obtained from the NIH-sponsored studies on recombinant DNA research. It was almost agreed that the original apprehensions on recombinant DNA research were unfounded.

It is a fact that the genetic engineering research flourished and progressed rapidly after relaxation of NIH guidelines. It may however be noted that NIH-RAC continues to be a watchdog over the DNA technology experiments.

Pharmaceutical products of recombinant DNA

As the recombinant DNA technology progressed, many pharmaceutical compounds of human health care are being produced through genetic manipulations. Most countries consider that the existing regulations for approval of pharmaceuticals of commercial use are adequate to ensure safety since the process by which the product manufactured is irrelevant. Thus, the recombinant DNA product (protein, vaccine, drug) is evaluated for its safety and efficacy like any other pharmaceutical product.

Genetically engineered organisms (GEOs)

Recombinant DNA research has resulted in the creation of many genetically engineered organisms. These include microorganisms, animals and plants. The latter two respectively result in transgenic animals and transgenic plants. The safety aspects and other related matters of GEOs are discussed at appropriate places in text, and some major highlights are given in Chapter 61.

THE FUTURE OF GENETIC ENGINEERING

DNA technology has largely helped scientists to understand the structure, function and regulation of genes. The development of new/modern biotechnology is primarily based on the success of DNA technology. Thus, the ***present biotechnology (more appropriately molecular biotechnology) has its main roots in molecular biology.***

Biotechnology is an interdisciplinary approach for applications to human health, agriculture, industry and environment. The major objective of biotechnology is to solve problems associated with human health, food production, energy production and environmental control.

The major contributions of genetic engineering through the new discipline biotechnology are given in this book.

It is an accepted fact that the recombinant DNA technology has entered the main stream of human life and has become one of the most significant applications of scientific research. ***Biotechnology is regarded as more an art than a science.***

After the successful sequencing of human genome, many breakthroughs in biotechnology are expected in future.