Chapter 64

# Biotechnology & Genetic Engineering

## BIOTECHNOLOGY

- Biotechnology means **any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use**.
- Biotechnology is an **important tool used for the production of food crops, livestock management, human health care, chemical industries and environmental management**.
- "Biotechnology" **generally refers to recombinant DNA based and/or tissue culture based processes** that have only been commercialized since the 1970s.
- One aspect of biotechnology is the direct use of organisms for the manufacture of organic products (examples include beer and milk products).
- Biotechnology is also used to recycle, treat waste, clean up sites contaminated by industrial activities **(bioremediation)**, and produce biological weapons.
- There are also applications of biotechnology that do not use living organisms. Examples are DNA microarrays used in genetics and radioactive tracers used in medicine.
- **Red biotechnology** is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genomic manipulation.
- **White biotechnology**, also known as **grey biotechnology**, is biotechnology applied to industrial processes.
- **Green biotechnology** is biotechnology applied to agricultural processes. An example is the designing of transgenic plants to grow under specific environmental conditions or in the presence (or absence) of certain agricultural chemicals.
- **Applications of biotechnology** are –
  - **Food and dairy products :** Yoghurt, cheese and butter require specific strains of micro-organisms during their preparation.
  - **Alcoholic and non-alcoholic beverages :** Beers, wines, etc. are produced through fermentation of different food by suitable micro-organisms. Curing of coffee beans and tea leaves is also a microbial process.
  - **Biofertilizers :** They are nitrogen fixing micro-organism which may live freely in soil or in association with plants.
  - **Organic acid :** A number of organic acids (acetic acid, lactic acid, etc.) are obtained through biotechnology.
  - **Vitamins :** Some vitamins are still manufactured with the help of micro-organisms. Food yeast is rich in both proteins and vitamins.
  - **Antibiotics :** Barring a few, all others are products of micro-organisms.
  - **Vaccines :** These contain attenuated or killed pathogens or their antigens.
  - **Monoclonal antibodies :** Antibodies against pathogen can now be obtained in pure form from clonal cultures.
  - **Hormones :** Insulin, growth hormone and other hormones are presently synthesized through the use of microbes and genetic engineering.
  - **Tissue culture :** An important tool for improvement of agriculture, forestry, synthesis of specific biochemicals etc.
  - **Genetic engineering :** Recombinant DNA technology is applied to several

biotechnological processes in obtaining particular biochemicals, improvement of genetic make–up of an organism and fighting undertaken for production of offspring of desired parents.

– **Steroids :** Micro-organisms are employed for transformation of one type of steroids into other types. These are required in antifertility formulations.

- **Yeast**, (*Saccharomyces cerevisiae*) is **the most important and most extensively used micro-organism** in **biotechnology**.
- Yeast cells are used in the manufacture of bread and also as a source of food, vitamins and other growth factors.
- There are **two types of yeasts: baker's yeast** and **alcohol yeast**.
- **Baker's yeast** is **grown on molasses** and sold as a food flavouring agent.
- **Alcohol yeast** is used by brewing industry for the production of different types of alcoholic beverages depending upon the medium and fermenting agent.
- There are two types of fermentation process: **batch fermentation** (or **closed system**) and **continuous process** (or **open system**).
- **Downstream processing** is the name given to the **stage after fermentation** when **desired product** is **recovered and purified**.
- **Cheese** is prepared by the **coagulation of casein** and other minor milk proteins (curdling of milk) by an **enzyme rennin extracted from calf gastric mucosa**.
- *Streptococcus* and *Lactobacillus* species are involved in the manufacture of most cheese.
- In cheese manufacture, micro-organisms are important in both souring and ripening processes.
- Semisoft blue **Roquefort cheese** of France is produced using the mold *Penicillium roqueforti*.
- **Yoghurt** is a preserved milk product having a distinct taste and a thick texture than milk.
- Yoghurt is made by fermenting whole milk with a mixture of *Lactobacillus bulgaricus, Streptococcus lactis* and *S. thermophilus* at 40º to 46ºC.
- **Gibberellins**, a plant growth hormone or phytohormone is obtained from a fungus called *Fusarium moniliformae* (or *Gibberella fujikuroi*). This was isolated by **Yabuta and Sumuki** (1939). At this time, over 52 gibberellins have been discovered from different plants.
- Louis Pasteur found that beer is produced by activity of yeast and yeast like micro-organisms. Yeast species used in alcoholic fermentation are *Saccharomyces cerevisiae* (Brewer's yeast), *S. ellopsoidens* (Wine yeast), *S. sake* (Sake yeast) and *S. pireformis* (Ginger beer/Ale yeast).
- **Acetic acid** is the most important organic acid used in industry.
- **Vinegar** is the **product resulting from the conversion of ethyl alcohol to acetic acid** by the bacteria *Acetobacter*.
- **Lactic acid** was the **first acid to be produced** microbially by *Lactobacillus delbrueckii*.
- *Aspergillus niger* and *Mucor* sp and yeast can ferment sugar to produce **citric acid**. It is used in medicine, flavouring extracts food and candies, manufacturing of ink, dyeing and engraving.
- **Gallic acid** is obtained using *Aspergillus niger.*
- **Gluconic acid** is manufactured with the help of *Penicillium purpurogenum* and *P. chrysogerium*. It is used in pharmaceuticals. **Calcium gluconate is used as a source of calcium in feeding infants** and **pregnant women** and **for treatment of milk fever in high producing dairy cows**.
- **Dextran** is a **plasma expander used in blood transfusions**.
- Dextran is a **complex polysaccharide prepared either through partial hydrolysis of starch or polymerization of sucrose** by the bacterium *Leuconostoc mesenteroides.*
- Some important enzymes produced industrially by microbes are **amylase**, **glucoamylase**, **glucose isomerase** and **proteases**.
- **Amylase** which attacks starch is **used in the manufacture of beer**, **bread and textiles**.
- Glucose can be attacked by **glucose isomerase** to **produce fructose corn syrup** which is sweeter than either glucose or sucrose and is **used in the production of soft drinks** and in **baking industry to sweeten biscuits and cakes**.
- The enzyme **Tissue Plasminogen Activator** (TPA) is **used for dissolving blood clots**.
- The technique of anchoring an enzyme in or on support material is called **immobilization**.
- Immobilized enzymes are generally used for bioreactors in a continuous process.
- **Cross-linking of enzymes** involves the chemical

reaction of the amino group of enzyme protein with glutaraldehyde.

- The **bonding** of enzyme can be done through **adsorption**, **ionic bonding or covalent bonding**.
- **Enzyme inclusion** involves the incorporation of enzyme into a semipermeable membrane.
- Most of the **vitamins** are made **commercially by chemical synthesis**.
- **Vitamin C** was the **first vitamin to be produced** by a fermentation process using *Acetobacter,* a wild bacterium.
- Bacteria used for industrial production of **vitamin $B_{12}$** are *Propionibacterium shermanii, P. frendenreichii* and *Pseudomonas denitrificans*.
- **Vitamin $B_2$** (Riboflavin) is synthesized by many micro-organisms including bacteria, yeasts and fungi. The fungus, *Ashbya gossypii* is **used for the microbial production of vitamin $B_2$**.
- **Antibiotics** are chemical substances produced by certain micro-organisms that kill or inhibit the growth of other micro-organisms.
- **Alexander Fleming, Howard Florey** and **Ernst Boris Chain** received **Nobel Prize** in 1945 **for the discovery and development of Penicillin**.
- **Streptomycin** was first isolated by **Selman A. Waksman**.
- **Gram-positive bacteria** are **usually more sensitive to antibiotics** than Gram-negative bacteria.
- An antibiotic that acts on a variety of pathogenic organisms is called a **broad spectrum antibiotic**.
- **Vaccination** or administration of vaccine was **discovered by Edward Jenner** (1796) when he immunised a boy against small pox by inoculating him with milder cow pox. The technique of attenuating or weakening of pathogen was

**Table :** Types of antibiotic with their source and action

| Antibiotics | Source | Action |
|---|---|---|
| Penicillin | *Penicillium chrysogenum*, *P. notatum* + Phenyl Acetic Acid | Tonsilitis, Sore Throat, Gonorrhoea, Rheumatic Fever, some Pneumonia types |
| Griseofulvin | *Penicillium griseofulvum* | Antifungal, especially for Ringworm |
| Nystatin | *Streptomyces noursei* | Antifungal for Candidiasis and overgrowth of Intestinal Fungi during excessive antibiotic treatment. |
| Hamycin | *Streptomyces pimprei* | Antifungal for Thrush |
| Fumagillin | *Aspergillus fumigatus* | Broad spectrum antibacterial especially against *Salmonella* and *Shigella*. |
| Bacitracin | *Bacillus licheniformis* | Syphilis, Lymphonema or Reticulosis. |
| Streptomycin | *Streptomyces griseus* | Meningitis, Pneumonia, Tuberculosis and Local Infections. Toxic in some through eighth cranial nerve. |
| Chloramphenicol Chloromycetin | *Streptomyces venezyelae, S. lavendulae,* Now synthetic | Typhoid, Typhus, Whooping cough, Atypical Pneumonia, Bacterial Urinary Infections |
| Tetracyclines/ Aureomycin | *Streptomyces aureofaciens* | Viral pneumonia, Osteomyelitis, Whooping Cough. Eye infections. |
| Oxytetracycline/ Terramycin | Chlorotetracycline → Hydrogenation *Streptomyces rimosus* | Intestinal and Urinary Infections (Spirochaetes, Rickettsiae, Viruses) |
| Erythromycin | *Streptomyces erythreus* (= *S. erythraeus*) | Typhoid, Common Pneumonia, Diphtheria, Whooping Cough, etc. |
| Gentamycin | *Micromonospora purpurea* | Effective against Gram (+) bacteria |
| Polymixin | *Bacillus polymyxa* | Antifungal |

discovered by Louis Pasteur (1879, against cholera).

- **Vaccines** are suspensions or killed or modified pathogenic micro-organisms; when injected into an animal, these produce immunity to a particular disease.
- Biotechnology has proved successful in the development of recombinant vaccines also known as '**second-generation vaccines**' and even **'third-generation vaccines'** (synthesized vaccines).
- **Hepatitis B vaccine is** a **second generation vaccine**.
- Vaccines produced using genetic engineering can usually be made faster than those manufactured by traditional methods.
- Genetically engineered vaccines are safer, more reproducible and can be administered in high doses without fear of side effects.
- **Monoclonal antibodies** are made outside the body by the hybrid cell cultures known as **hybridomas**.
- The cells obtained from cancerous tumors are known as **myeloma**.
- B-lymphocytes are mixed with myeloma cells resulting in hybridoma.
- The hybridoma cells are identified when all cells are grown in a medium deficient in the nutrient needed by myeloma cells.
- To produce monoclonal antibodies, B-cells are removed from the spleen of an animal that has been challenged with the relevant antigen.
- These B-cells are then fused with myeloma tumor cells that can grow indefinitely in culture (myeloma is a B-cell cancer).
- This fusion is performed by making the cell membranes more permeable.
- The fused hybrid cells (called hybridomas), being cancer cells, will multiply rapidly and indefinitely and will produce large amounts of the desired antibodies.
- They have to be selected and subsequently cloned by limiting dilution. Supplemental media containing Interleukin-6 (such as briclone) are essential for this step.
- Monoclonal antibodies are true **magic bullets**, striking specific molecules and leaving the rest of the body unharmed.
- Monoclonal antibodies (mAb) are antibodies that are identical because they were produced by one type of immune cell and are all clones of a single parent cell.
- One clinical application of monoclonal antibodies is **immune suppression for kidney transplantation**.
- Monoclonal antibodies have also been used in **genetics engineering for identifying and measuring levels of gene products not detectable by other methods**.
- Monoclonal antibodies are **used in pregnancy testing**, **diagnosis of disease, treatment of disease, preventing rejection of transplants and tissue typing for transplants**.
- They are **also very useful in immunohistochemistry** which detect antigen in fixed tissue sections. Monoclonal antibodies can also be used to purify a substance with techniques called immunoprecipitation and affinity chromatography.
- One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immunological response against the target cancer cell.
- Such mAb could also be modified for delivery of a toxin, radioisotope, cytokinin or other active conjugate; it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell.
- In fact, every intact antibody can bind to cell receptors or other proteins with its Fc region.
- **Insulin** is a protein consisting of two short polypeptide chains **A** and **B** of 21 and 30 amino acids respectively interconnected by two **disulphide bridges**.
- Insulin **regulates sugar metabolism and insufficiency or lack of insulin leads to high blood sugar level** and the disease is called **diabetes**.
- **Banting and Best** (1921) isolated insulin from pancreas of dog and demonstrated its efficacy in curing diabetes in humans.
- In 1983, 5 July, American Company Eli Lilly prepared two DNA sequences corresponding to A and B insulin chains when introduced in plasmids of *E. coli*, insulin chains were formed. They were extracted and fused to produced **humulin (human insulin)**.
- **Growth hormone** is required to overcome pituitary dwarfism caused by nonsecretion of hormone from anterior pituitary.
- The DNA/gene cDIVA (without introns) required for synthesis of growth hormone is first synthesized

and integrated with bacterial plasmid (trp gene). The latter produces the required hormone. It has been also been produced produced inside silk worm.

- **Interferons** (antiviral proteins) were produced by Charles Weismann of Zurich University through recombinant-DNA technology in *E. coli* in 1980.
- **Transgenic plants** are plants with specific genes or traits obtained through genetic engineering of DNA recombinant technology, *e.g.*, resistance to hornworm larvae (tomato), resistance to corn borer (Bt corn with gene from Bacillus *thuringiensis*), resistance to over-ripening (tomato), good protein content (potato), herbicide resistance (tobacco).
- **Transgenic animals** are animals having specific genes obtained from outside, *e.g.*, tissue plasminogen activator in milk (goat), blood clotting factor VIII (sheep from progeny of ewe Eithel).
- Cowdung, farm refuse, garbage etc are placed in biogas plants where anaerobic conditions allow methane bacteria to produce methane and other fuel gases called as biogas. The organic remains of the biogas plants are used as manure.
- **Biofertilizers** are mostly nitrogen fixing micro-organisms which may live free in the soil or form associations with plants. Special strains of these organisms are now inoculated to soil or seeds.
- The main technique involved in agricultural biotechnology is called **tissue culture**.
- The medicinally important plant products can be manufactured on a commercial scale by using cell and tissue culture techniques.
- Recombinant DNA technology is applied to several biotechnological process in obtaining particular biochemicals, improvement of genetic make up of an organism and fighting genetic defects.
- **Test tube babies and embryo transplants** are now being routinely undertaken for production of offspring of desired parents.
- **Steroids** are complex crystalline lipids having tetracyclic hydrocarbon core, with one 5-carbon ring and three 6-carbon rings.
- Most steroid bioconversions involve **hydroxylation** and **a variety of different fungi are used industrially to carry out one or another specific hydroxylations**.
- **Four major steroids** currently produced by bioconversion are **hydrocortisone, cortisone, prednisone** and **prednisolone**.
- Some microbes used in microbial transformations of steroids are *Rhizopus nigricans, R. arrihizus, Cunninghamella blakesleeana, Curvularia lunata* and *Corynebacterium simplex*.
- Steroids are **used medicinally in correcting hormonal imbalance**, as **anabolic stimulants, birth control pills** (progesterone ± estrogen), **antifertility drugs** (*e.g.*, diosgenin), **anti-inflammatories**, **for relieving pain** and **suppressing immune responses**.

## Biopatent, Biopiracy and Biowar

- A **patent is a monopoly granted to a person** who has either invented a new and useful article, made and improvement of an existing article or invented a new process of making an articles.
- A patent is granted by the legal system, therefore it is a subject which cannot be fully understood without knowing the law on the subject.
- Biopatents are awarded for the following as strains of micro-organisms, cell lines, genetically modified strains of plants and animals, DNA sequences, the proteins encoded by DNA sequences, various biotechnological procedures, production processes, products and product applications.
- The **human breast cancer gene** (BRCA1) was patented in the US once its base sequence had been determined and attempts are being made to patent the second breast cancer gene (BRCA2).
- **Biopiracy** (or **biocolonialism**) is the appropriation of another's knowledge of use of biological resources.
- **Intellectual Property Right** (IPR) claims by the formal sector over the work of the informal constitutes biopiracy.
- Three **aspects of biopiracy** are –
  - **Intellectual Piracy:** This makes a false claim to novelty and invention, even though the knowledge has evolved since ancient time.
  - **Resource Piracy:** This divests scarce biological resources to monopoly control of corporations thus depriving communities and indigenous practitioners.
  - **Economic Piracy:** It creates market monopolies and excludes the original innovators from their rightful share to local, national and international markets.

- Neem, *Azadirachta indica,* has been used by the people of India in a variety of ways for time immemorial.
- The patenting of the fungicidal properties of neem was an example of biopiracy.
- An American university patented the healing properties of **turmeric powder,** cherished in India since ancient times for its powder to cure the wounds.
- **Biological resources** or **bioresources** include all those organisms that can be used to derive commercial benefits.
- Traditional knowledge related to bioresources is the knowledge developed by various communities over long periods of history, regarding the utilization of the bioresources, *e.g.,* use of herbs as drugs.
- **Biowar** or **biological war** or **bioterrorism** is the deployment of biological weapons against people, their crops and animals.
- A biological weapon or bioweapon **carries and delivers to the target organism a pathological biological agent or a toxin derived from it**.
- Mass-produced pathogens or their toxins are delivered either as powder or in the form of spray, using a variety of delivery devices.
- Among weapons of mass destruction, biological weapons are more destructive than chemical weapons including nerve gas.
- Bioweapons (a) are low-cost weapons, (b) cause far more casualities than chemical or conventional weapons, and (c) bioweapon agents are invisible and extremely difficult to detect.
- The first reported use of biological weapons was in 5th century BC, when **Assyrians** poisoned enemy wells with rye ergot.
- During many occasions, **smallpox** was used as a biological weapon.
- Iraq is reported to have conducted research and development work on anthrax, botulin, aflatoxin, wheat cover smut and ricin.
- Anthrax spores were used against USA and her allied countries by Al-Qaeda activists.
- Various poisons produced by different biological pathogens act like some neurotoxin, such as **saxitoxin** can kill individuals by blocking nerve conduction directly.
- The possible defences against bioweapons include the use of respirator or gas mask, vaccination, administration of appropriate antibiotics and decontamination.
- Biological warfare agents include –
  - **Pathogens :** Smallpox virus, Viral encephalitides, Viral haemorrhagic fevers, *Bacillus anthracis*, *Brucella suis*, *Coxiella burnetii*, *Francisella tularensis*, *Yersinia pestis*.
  - **Toxins :** Botulinum, Ricin, Stylococcal enterotoxin B.
  - **Anticrop agents :** Rice blast, Rice stem rust, wheat stem rust.
- **Bioethics** is the branch of ethics, philosophy and social commentary that deals with the biological sciences and their potential impact on society.
- The **major bioethical concerns pertaining to biotechnology** are –
  - Use of animals in biotechnology causes great suffering to them.
  - When animals are used for production of pharmaceutical proteins, they are virtually reduced to the status of a 'factory'.
  - Introduction of a transgene from one species into another species violates the 'integrity of species'.
  - Transfer of human genes into animals (and *vice-versa*) dilutes the concept of 'humanness'.
  - Biotechnoloy is disrespectful to living beings and only exploits them for the benefit of human beings.
  - Biotechnology may pose unforeseen risks to the environment, including risk to biodiversity.
- **Bioinformatics** is an interdisciplinary field which addresses biological problems using computational techniques.
- The field is also often referred to as computational biology.
- It plays a key role in various areas, such as functional genomics, structural genomics, and proteomics, and forms a key component in the biotechnology and pharmaceutical sector.

## GENETIC ENGINEERING

- Genetic engineering is the **technology involved in synthesis of artificial genes, repair of genes through fusion, deletion, inversion, shifting of genes, products of recombinant DNA & manipulating them** for improvement in human

beings, plants, animals and microbes.

- Genetic engineering is the **most powerful technique available in applied genetics**.
- An **important aspect** of genetic engineering is **recombinant DNA technology**.
- Recombinant DNA technology is **employed for** combining **DNA from two different organisms to produce recombinant DNA**.
- The process involves the following steps –
  - Separation of a desired DNA segment from donor organism
  - Selection of suitable vector
  - **Cutting DNA into specific fragments** using enzyme restriction endonuclease and **joining the fragments with the help of enzyme ligase**.
- The technology of genetic engineering came into existence after the **introduction of genes of SV-40 into the bacterium with the help of lambda phage (Berg 1970s)**.
- **Paul Berg** is the **father of genetic engineering** (Nobel Prize, 1980).
- **First nonfunctional artificial gene** of alanine *t* RNA having 77 nucleotides pairs was synthesized by **H.G.Khorana *et al* (1968)**.
- **First functional artificial gene** was tyrosine *t*RNA gene with 207 nucleotide pairs (H.G.Khorana *et al* 1971).
- In 1981, **Edge *et al*** synthesized **IFN-$\alpha$ gene** (human leucocyte interferon gene) with 514 base pairs.
- Addition of DNA ligases produces **recombinant DNA**.
- The technology associated with the construction and application of **recombinant DNA** (which is generated *in vitro* by covalently joining DNA molecules from different sources) is referred to as **genetic engineering** or **gene splicing** or **gene manipulation**.
- **Enzymes used in genetic engineering** to perform specific functions are –
  - **Restriction endonuclease** (cut DNA at specific sites)
  - **DNA ligase** (join the cut DNA)
  - **Exonuclease** (digest the base pairs on 5′ or 3′ end of a single stranded DNA or at single stranded nicks or gaps in double stranded DNA)
  - **Endonuclease** (cleave the double stranded DNA at any point except the ends)
  - **DNA polymerase** (polymerises the DNA synthesis on DNA template)
  - **Reverse transcriptase** (used to synthesize cDNA by using *m*RNA template).
- **Vectors** (also known as **vehicle DNAs**) are those DNA molecules that can carry a foreign DNA fragment when inserted into it.
- The vectors are grouped into **bacterial plasmids**, **bacteriophage**, **cosmids** and **phasmid**.
- **The vehicle DNA carrying passenger DNA is called recombinant DNA (chimeric DNA)**.
- **Plasmids** (like $p^{BR322}$, $p^{BR324}$, $p^{C194}$ etc) are the extrachromosomal, self-replicating and double stranded closed and circular DNA molecules present in the bacterial cell.
- **Cohen *et al*** (1973) for the first time reported the cloning DNA by using plasmid as vector.
- Bacteriophages (a virus that eats upon bacteria) are **required for cloning of large DNA fragment**.
- The **cosmids** (like $p^{JC74}$, $p^{JC720}$ etc.) can be defined as the **hybrid vectors derived from plasmids which contain *cos* site of phage l**.
- A phage genome containing *att* site and one or more plasmid molecule (s) is known as **phasmid**.
- One use of transgenic animals is to produce relatively large quantities of rare and expensive proteins for use in medicine, a process sometimes referred to as '**pharming**' of drugs.
- Detecting mutant genes in an individual is known as **genetic screening**.
- Genetic screening **reduces suffering of both victims of genetic disease** (Thalassemia, sickle cell anaemia) and their families.
- **Eugenics** is the study of the possible improvement of the genetics of a species.
- **Genetic counselling** is the advice given to a couple or prospective couple or their families about the possibility of genetic disorders in the future (unconceived) baby and in an early foetus as an aid to decision making about marriage or having children. Advice is given by physicians or professional human geneticists. They are called **genetic counsellors**.
- Genetic engineering techniques give a scope to learn a great deal about the **human genome**.

- The human genome contains some **3.2 billion bases**.
- **Genome sequencing** has revealed that organisms have many genes in common. Surprisingly, humans have a small genome, only a third larger than nematode worm.
- **Proteomics**, the next step of human genome project deals with cataloguing and analysis of every protein in the human body.
- A **biochip** is a discrete collection of gene fragments on a stamp-sized chip that can be used to screen for the presence of particular gene variants.
- Biochips allow rapid screening of gene profiles, a tool that promises to have a revolutionary impact on medicine and society.
- Biochips can **help in identifying precise forms of cancer**.
- **Gene therapy** is the treatment of disease by replacing, altering, or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for the disease. Gene therapy is unique in that it employs the genetic material, DNA, itself as the means of treatment.
- During gene therapy, DNA that codes for specific genes is delivered to individual cells in the body.
- Gene therapy is **being used in many ways**. For example, to:
  - Replace missing or defective genes
  - Deliver genes that speed the destruction of cancer cells
  - Supply genes that cause cancer cells to revert back to normal cells
  - Deliver bacterial or viral genes as a form of vaccination
  - Provide genes that promote or impede the growth of new tissue
  - Deliver genes that stimulate the healing of damaged tissue.
- A large variety of genes are now being tested for use in gene therapy. *Examples include :* a gene for the treatment of cystic fibrosis (a gene called **CFTR** that regulates chloride); **genes for factors VIII and IX**, deficiency of which is responsible for classic haemophilia (haemophilia A) and another form of haemophilia (haemophilia B), respectively; genes called **E1A** and **P53** that cause cancer cells to undergo cell death or revert to normal; **AC6** gene which increases the ability of the heart to contract and may help in heart failure; and **VEGF**, a gene that induces the growth of new blood vessels (angiogenesis) of use in blood vessel disease.

## Cloning

- Cloning is the production of copies that are genetically identical to the parent.
- Cloning is **absent in higher animals except** for occasional monozygotic split ups.
- Cloning is of **three types – cell cloning, gene cloning** and **organismal cloning**.
- **Cell cloning** is the formation of multiple copies of the same cell. Cells of a clone are identical genetically, morphologically and physiologically.
- **Totipotency** or ability to divide indefinitely and differentiate into full fledged organisms is present in most plants cells. In case of animals, it is found only in zygote (fertilized egg) and embryonic stem cells.
- Animal cells can **have pleuripotency or potential ability to develop into any other type of cell in the animal body,** *e.g.*, kidney cells, heart cells, liver cells, nerve cells.
- **HeLa cells** are **aneuploid strain of cell lines** from cervix carcinoma of a patient Henrietta Lacks which have been grown continuously since 1952. They have been used in the study of various life processes including infection by viruses.
- **Hybrid cells** are obtained through protoplast fusion of human and mouse cells (first carried out by Burski *et al*, 1960). Hybrid cells can be made to grow and divide indefinitely under proper culture conditions. The **cells are used for mapping genes, study of cancer formation and regulation of gene expression**.
- Recently techniques are being perfected to grow full fledged organs from small pieces containing stem cells and organ cells. Pig organs can also be used in organ transplantation provided they are genetically modified to prevent rejection from human immune system. This technique is called **organ culture**.
- **Gene cloning** is the formation of multiple copies of same gene.
- It is achieved by recombinant DNA technology. The steps include –
  - The production of a lineage of cells all of which contain one kind of DNA fragment of

interest derived from a population of many kinds of DNA fragments.
  - Operational by inserting (recombining) a population of DNA molecules.
  - Known to contain the DNA of interest, into a population of vector DNA molecules in such a way that each vector molecule contains only a single DNA molecule from the original population.
  - Transforming a population of host cells with the vector DNA recombinants such that each host cell takes up only one vector.
  - Growing single host cells separately (cloning) by plating at low density to form a collection of separate colonies.
  - Screening the colonies (clones) formed for the presence of the DNA of interest.
- **Organismal cloning** is the formation of one or more genetically identical individual from a single parent.
- The lamb **Dolly** is the **first successful clone** derived from differentiated animal cell **(Wilmut and Campbell 1997)**.
- They took cells from the udder of a six year old sheep. The cells were arrested in $G_0$ – phase by serum starvation. Unfertilized egg of another adult sheep was taken out when it was receiving optimum amount of maturation promoting factors. The egg was denucleated. Nondividing nucleus of an udder cell was taken out and inserted in the denucleated egg. In nutrient medium the egg began to undergo cleavage. The young embryo was implanted in the womb of a third sheep. The surrogate mother gave birth to normal healthy lamb, Dolly, on February 13, 1997.
- In **molecular cloning**, the DNA fragment of interest is amplified *in vivo* in a population of proliferating cells.
- A **cloning vector** is a genetic element derived from a plasmid or virus which is exploited to carry extra DNA (**donor**, **foreign**, **insert** or **passenger DNA**).
- Crown gall producing bacterium, *Agrobacterium tumefaciens*, possesses tumor inducing or Ti plasmids. The plasmids pass the tumor producing gene into the genome of the host plant. The transformed host genome produces galls. Because of this *Agrobacterium* functions as **natural genetic engineer of plants**.
- The modified plasmid of *Agrobacterium* is made to carry the desired gene. It is called **T-DNA**. The same can be directly inserted into nucleus of host plant cells.
- Alternately T-DNA is also made to carry a gene for antibiotic resistance.
- *Agrobacterium* mediated gene cloning has been carried out in many dicotyledonous plants (*e.g.*, sunflower, cotton, potato, tomato) as well as all the major cereals recently (*viz.* wheat, maize, rice).
- The animal, derived from a mixture of two genetically different types of cells, some derived from the normal stem cells and some derived from the transformed stem cells, is known as **chimera**.
- **Gene bank** or **genomic library** is a complete collection of cloned DNA fragments which comprises the entire genome of an organism.
- **Gene banks** are a means of preserving genetic material, be it plant or animal. In plants, this could be freezing the plant, or the seeds themselves.
- In plants, it is possible to unfreeze the material and sow it, however, in animals, a living female is required for artificial insemination.
- Despite this, it has proven very difficult to utilize the genes after freezing.

## Techniques of genetic engineering

### PCR

- **Polymerase chain reaction** (**PCR**) is a biochemistry and molecular biology technique for enzymatically replicating DNA without using a living organism, such as *E. coli* or yeast.
- Like amplification using living organisms, the technique allows a small amount of DNA to be amplified exponentially.
- PCR can amplify specific sequences or add sequences (such as endonuclease recognition sequences) as primers to cloned DNA.
- The **enzyme used in PCR** is **Taq polymerase**.
- As PCR is an *in vitro* technique, it can be performed without restrictions on the form of DNA and it can be extensively modified to perform a wide array of genetic manipulations.
- PCR is **commonly used in medical and biological research labs for a variety of tasks,** such as the

**Table :** Products from genetically engineered microbes

| | | |
|---|---|---|
| 1. | *Escherichia coli* (gut bacterium) | Human insulin, human growth factor, interferons, interleukin, tissue plasminogen activator, epidermal growth factors, lung surfactant protein, factor VIII, viral vaccines. |
| 2. | *Bacillus thuringiensis* (soil bacterium) | Endotoxin (Bt toxin), highly potent, safe and biodegradable insecticide. |
| 3. | *Rhizobium meliloti* (symbiont nitrogen fixing bacteria) | Transfer of 'Nif' genes to cereal crops. |
| 4. | *Pseudomonas fluorescence* (bacterium) | Prevents frost damage (e.g., Strawberry) on which it grows. Biochemicals extracted from bacterium also have similar effect. |
| 5. | *Pseudomonas putida* (bacterium) | Bioremediation or purification of environment – scavenging oil spills by digesting hydrocarbons, metabolism of heavy metals and other biochemicals. |
| 6. | *Trichoderma* (fungus) | Produces enzyme chitinase for biocontrol of fungal diseases in plants. |
| 7. | *Trametes* (fungus) | Removal of lignin from wood pulp. |

**detection of hereditary diseases, the identification of genetic fingerprints, the diagnosis of infectious diseases**, the **cloning of genes, paternity testing,** and **DNA computing**.

- The reaction is easy to execute.
- It requires no more than a test tube, a few simple reagents, and a source of heat."

### RAPD

- **RAPD stands for random amplification of polymorphic DNA**.
- It is a **type of PCR reaction,** but the segments of DNA that are amplified are random.
- The scientist performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify.
- No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is exactly not certain where.
- This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared (it is not suitable for forming a DNA databank).
- Due to the fact that it relies on a large, intact DNA template sequence, it has some limitations in the use of degraded DNA samples.
- Its resolving power is much lower than targeted, species specific DNA comparison methods, such as short tandem repeats.

### RFLP

- In molecular biology, the term **restriction fragment length polymorphism** (or **RFLP**, often pronounced "rif-lip") is used in two related contexts: as a characteristic of DNA molecules (arising from their different nucleotide sequences) by which they may be distinguished, and as the laboratory technique which uses this characteristic to compare DNA molecules.
- The technique is utilized in genetic fingerprinting and paternity testing.

### DNA fingerprinting or Genetic fingerprinting

- DNA finger printing is the technique in which the banding pattern of DNA fragments is compared and can be used in many species, including human, to indicate relatedness (used for rape victim, paternity, other criminals).
- **Dermatoglyphics** is the **science of finger printing** which was developed during a murder investigation in Jalpaiguri (WB) in 1897.
- It deals with the study of pattern of ridges of the skin of fingers, palms, toes and soles.
- Dermatoglyphics is **used in establishing identity of individuals**. It can **also indicate genetic abnormalities**.

- **More sensitive version of DNA finger printing is DNA profiling** (most commonly used method in forensic work).
- **Genetic fingerprinting**, **DNA testing**, **DNA typing**, and **DNA profiling** are **techniques used to distinguish between individuals of the same species using only samples of their DNA**.
- Its invention by Sir Alec Jeffreys at the University of Leicester was announced in 1985. Two humans will have the vast majority of their DNA sequence in common.
- Genetic fingerprinting **exploits highly variable repeating sequences called minisatellites** (VNTR's).
- DNA of each individual has some noncistronic hyper variable repeat minisatellite sequence. These repeat minisatellite sequence flanked by conserved restriction site are commonly called as (VNTRs) **variable number of tandem repeats**.
- **VNTRs** are **similar in twins only**.
- Two unrelated humans will be likely to have different numbers of minisatellites at a given locus.
- By using PCR enough DNA is obtained to detect the number of repeats at several loci.
- It is possible to establish a match that is extremely unlikely to have arisen by coincidence, except in the case of identical twins, who will have identical genetic profiles.
- Genetic fingerprinting is **used in forensic science,** to match suspects to samples of blood, hair, saliva or semen.
- It has also led to several exonerations of formerly convicted suspects.
- It is also used in such applications as identifying human remains, paternity testing, matching organ donors, studying populations of wild animals, and establishing the province or composition of foods.
- It has also been used to generate hypothesis on the pattern of the human diaspora in prehistoric times.
- Testing is subject to the legal code of the jurisdiction in which it is performed.

### Southern blotting

- Southern blotting is a method in molecular biology of enhancing the result of an agarose gel electrophoresis by marking specific DNA sequences.
- The method is named after its inventor, the British biologist Edwin Southern.
- This caused other blot methods to be named similarly as plays on Southern's name (for example, Western blot, Northern blot, Southwestern blot (Detection of RNA is termed northern blotting.).

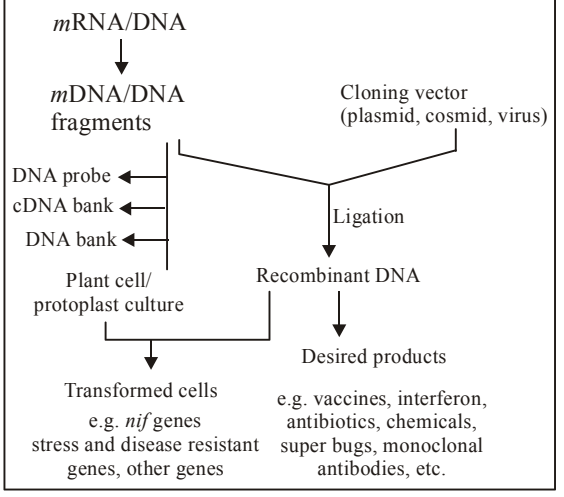
### Western blotting

- A western blot is a **method in molecular biology/ biochemistry/immunogenetics to detect protein in a given sample of tissue homogenate or extract**.
- It uses gel electrophoresis to separate denatured proteins by mass. The proteins are then transferred out of the gel and onto a membrane (typically nitrocellulose), where they are "probed" using antibodies specific to the protein.
- As a result, researchers can examine the amount of protein in a given sample and compare levels between several groups.
- Other techniques also using antibodies allow detection of proteins in tissues (immunohisto-chemistry) and cells (immunocytochemistry).
- The name **western blot** was given to the technique by **W. Neal Burnette (1981)**.

## Application of genetic engineering

- **Human insulin** or **humulin** is the first genetically engineered pharmaceutical product, developed by Eli Lilly and company in 1982.
- Genetech, a California-based company, have produced **human growth hormone** (hGH) from genetically engineered bacteria.
- **Somatostatin** is the first polypeptide, which was expressed in *E. coli* as a part of the fusion peptide.
- **BST** or **Bovine Somatotropin** is produced for a large quantity of milk production in cows.
- It is possible to cure **phenylketonuria** disease by using recombinant DNA techniques in early period of pregnancy.
- **Urokinase** is involved in dissolution of blood clots. It has been synthesized in huge quantity by using genetically engineered bacteria with urokinase genes.

**Outline of applications of genetic engineering techniques in different areas**




- **Human globin genes** has also been developed and cloned.
- By using gene cloning techniques the clotting factor VIII:C gene was cloned which expressed in mammalian cell lines and produced the protein VIII:C responsible for **blood clotting**.
- Recombinant DNA technology has helped in **increased production of antibiotics**; for example, the rate of penicillin produced at present is about 1,50,000 unit/ml against about 10 unit/ml in 1950s.
- A genetically engineered bacteria is capable of **cleaning up oil spills**.
- Organisms that have been genetically altered using the techniques of genetic engineering are generally referred to as **transgenic**.
- **Transgenic salmon** (a fish) is produced by inserting recombinant growth hormone genes into developing salmon embryos. They are 11 times heavier than non-transgenic salmon.
- **Weevil-proof peas, herbicide resistant crops** and **wilt-proof flowers** are produced by using genetic engineering.
- **Human enkephalin gene** has been expressed in plants thus producing medical drugs from crops instead of food.
- **Gene transfer** during transgenic plant formation includes (i) electroporation, (ii) particle bombardment (iii) micro injection, (iv) *Agrobacterium* - mediated gene transfer, (v) co-cultivation (protoplast transformation method), (vi) leaf disc transformation method, (vii) virus-mediated transformation, (viii) pollen-mediated transformation, (ix) liposome mediated transformation etc.
- In 1999, the Indian scientist at ICGEB, New Delhi have successfully produced transgenic maize, tobacco, rice etc. capable of producing **interferon gamma** (INF-γ).

End of the Chapter