

# 10

CHAPTER

## UNIT - IV

# Applications of Biotechnology



"Our world is built on biology"

### Chapter outline

- 10.1 Applications in Medicine
- 10.2 Gene therapy
- 10.3 Stem Cell Therapy
- 10.4 Molecular Diagnostics
- 10.5 Transgenic Animals
- 10.6 Biological products and their uses
- 10.7 Animal cloning
- 10.8 Ethical issues
- 10.9 Regulation in biotechnology
- 10.10 Possible threats of Genetically Modified Organisms
- 10.11 Biosafety guidelines



### Learning objectives

- Understand the applications of rDNA technology in the field of medicine.
- Analyse the role of diagnostic tools in Molecular diagnosis.
- Learn animal cloning and its applications.
- Create awareness on the ethical issues involved in biotechnology.
- Realise the need for biosafety guidelines and regulations in Biotechnology.



Before we start this chapter, it will be helpful if you revise the structure of DNA, Protein synthesis and genetic engineering. Genetic engineering involves the manipulation of DNA and naturally occurring processes such as protein synthesis for a wide range of applications including the production of therapeutically important proteins. This also involves extracting a gene from one organism and transferring it to the DNA of another organism, of the same or another species. The DNA produced in this way is referred to as recombinant DNA (rDNA) and this technique as recombinant DNA technology. All these are part of the broad field biotechnology which can be defined as the applications of scientific and engineering principles to the processing of material by biological agents to provide goods and services.

Biotechnology is an umbrella term that covers various techniques for using the properties of living things to make products or provide services. The term biotechnology was first used before 20<sup>th</sup> century for such traditional activities as making idli, dosa, dairy products, bread or wine, but none of these would be considered biotechnology in the modern sense.

In this chapter we will study the applications of bio-technology in various fields including the field of Medicine. Recombinant DNA technology has led to the large scale production of various hormones and proteins of therapeutic use.

## 10.1 Applications in Medicine

### 10.1.1 Recombinant Human Insulin

The Human insulin is synthesized by the  $\beta$  cells of Islets of Langerhans in the pancreas. It is formed of 51 amino acids which are arranged in two polypeptide chains, A and B. The polypeptide chain A has 21 amino acids while the polypeptide chain B has 30 amino acids. Both A and B chains are attached together by disulphide bonds. Insulin controls the levels of glucose in blood. It facilitates the cellular uptake and utilization of glucose for the release of energy. Deficiency of insulin leads to diabetes mellitus which is characterized by increased blood glucose concentration and a complex of symptoms which may lead to death, if untreated. A continuous program of insulin dependence is required to treat this deficiency.

In the early years, insulin isolated and purified from the pancreas of pigs and cows was used to treat diabetic patients. Due to minor differences in the structure of

the animal insulin as compared to human insulin, it resulted in the occurrence of allergic reactions in some diabetic patients. Production of insulin by recombinant DNA technology started in the late 1970s. This technique involved the insertion of human insulin gene on the plasmids of *E.coli*. The polypeptide chains are synthesized as a precursor called pre-pro insulin, which contains A and B segments linked by a third chain (C) and preceded by a leader sequence. The leader sequence is removed after translation and the C chain is excised, leaving the A and B polypeptide chains (Fig. 10.1).

Insulin was the first ever pharmaceutical product of recombinant DNA technology administered to humans. The approval to use recombinant insulin for diabetes mellitus was given in 1982. In 1986 human insulin was marketed under the trade name Humulin.

Best and Banting in 1921, isolated insulin from the pancreatic islets of a dog and demonstrated its effectiveness against diabetes.

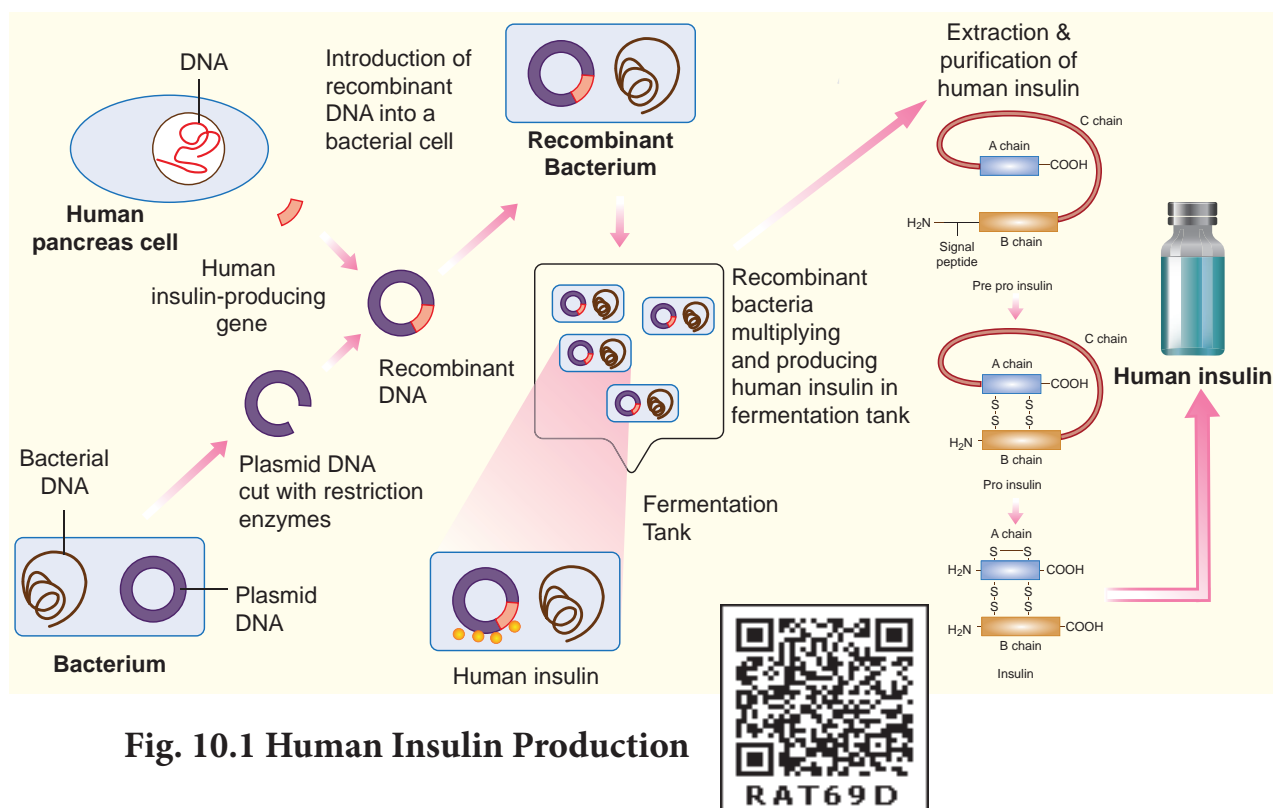


Fig. 10.1 Human Insulin Production

### 10.1.2 Human alpha lactalbumin

Alpha lactalbumin is a protein composed of 123 amino acids and 4 disulphide bridges, with a molecular weight of 14178 Da. In human milk,  $\alpha$  lactalbumin is the most abundant protein comprising 25% of total protein found in human milk. It is synthesized by the mammary glands.  $\alpha$  lactalbumin binds calcium and zinc ions and possesses bactericidal and anti tumour activities.

Improvement of the nutritional value of cow's milk with transgenic expression of recombinant human alpha lactalbumin has been attempted. Healthy transgenic cows were produced by somatic nuclear transfer, in which expression of upto 1.55g/L of recombinant human alpha lactalbumin was achieved. Similarly transgenic goats were also produced, in which the expression of Human alpha lactalbumin was about 0.1 to 0.9mg/mL.

Somatic cell nuclear transfer is a technique for creating a viable embryo from a body cell and an egg cell. This technique is discussed later in animal cloning.



In 1997, Rosie, the first transgenic cow produced human protein enriched milk, which contained the human alpha lactalbumin. The protein rich milk (2.4 gm/litre) was a nutritionally balanced food for new born babies than the normal milk produced by the cows.

### 10.1.3 Interferons

Interferons are proteinaceous, antiviral, species specific substances produced by mammalian cells when infected with viruses. Interferons were discovered by Alick Isaacs and Jean Lindemann in 1957. Based on the structure of interferons they are classified as  $\alpha$ ,  $\beta$  and  $\gamma$  interferons. They stimulate the cellular DNA to produce antiviral enzymes which inhibit viral replication and protect the cells. Interferons

could be isolated from blood, but the amount of blood required for isolation of interferons is enormous and not practical. To overcome this issue interferons could be produced by rDNA technology. The yeast *Saccharomyces cerevisiae* is more suitable for production of recombinant interferons than *E.coli*, since *E.coli* does not possess the machinery for glycosylation of proteins. Interferons are used for the treatment of various diseases like cancer, AIDS, multiple sclerosis, hepatitis C and herpes zoster. In spite of the therapeutic applications interferons are not within the reach of the common man due to high cost for its production.

### 10.1.4 Recombinant Vaccines

Recombinant DNA technology has been used to produce new generation vaccines. The limitations of traditional vaccine production could be overcome by this approach.

The recombinant vaccines are generally of uniform quality and produce less side effects as compared to the vaccines produced by conventional methods. Different types of recombinant vaccines include subunit recombinant vaccines, attenuated recombinant vaccines and DNA vaccines.

#### Subunit recombinant vaccines

Vaccines that use components of a pathogenic organism rather than the whole organism are called **subunit vaccines**; recombinant DNA technology is very suited for developing new subunit vaccines. It includes components like proteins, peptides and DNAs of pathogenic organisms. The advantages of these vaccines include their purity in preparation, stability and safe use.

#### Attenuated recombinant vaccines

This includes genetically modified pathogenic organisms (bacteria or viruses) that are made nonpathogenic and are used as vaccines. It is now possible to genetically engineer the organisms (bacteria or viruses) and use them as live vaccines and such vaccines are referred to as attenuated recombinant vaccines.

Edible vaccines are prepared by molecular pharming using the science of genetic engineering. Selected genes are introduced into plants and the transgenic plants are induced to manufacture the encoded protein. Edible vaccines are mucosal targeted vaccines which cause stimulation of both systemic and mucosal immune response. At present edible vaccines are produced for human and animal diseases like measles, cholera, foot and mouth disease and hepatitis.

### DNA Vaccines

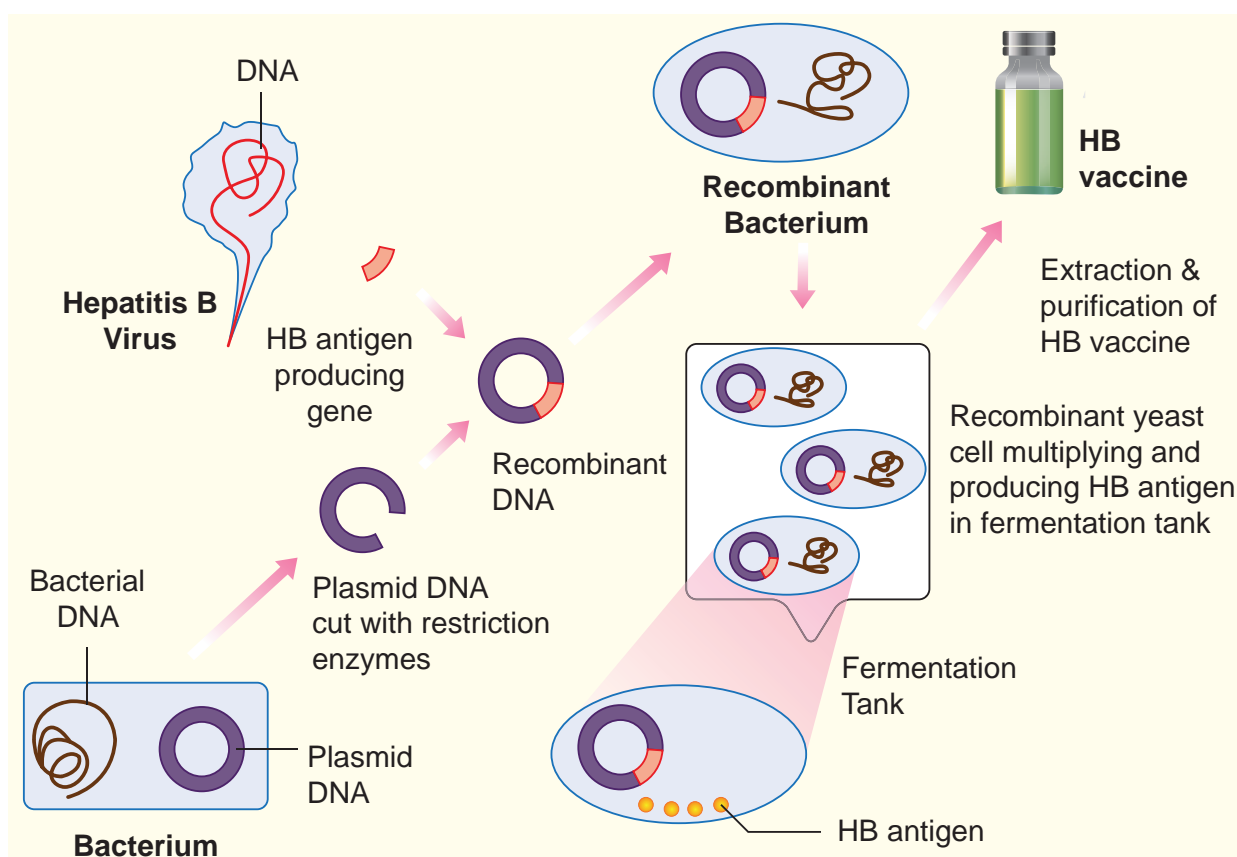
Genetic immunisation by using DNA vaccines is a novel approach that came into being in 1990. The immune response of the body is stimulated by a DNA molecule. A DNA vaccine consists of a gene encoding an antigenic protein,

inserted onto a plasmid, and then incorporated into the cells in a target animal. DNA instructs the cells to make antigenic molecules which are displayed on its surfaces. This would evoke an antibody response to the free floating antigen secreted by the cells. The DNA vaccine cannot cause the disease as it contains only copies of a few of its genes. DNA vaccines are relatively easy and inexpensive to design and produce.

Vaccines produced by these new techniques have definite advantages like producing target proteins, long lasting immunity and trigger immune response only against specific pathogens with less toxic effects.

Recombinant hepatitis B vaccine as a subunit vaccine is produced by cloning hepatitis B surface antigen (HbsAg) gene in the yeast, *Saccharomyces cerevisiae* (Fig. 10.2).

The recombinant vaccine for hepatitis B (HbsAg) was the first synthetic vaccine launched in 1997 which was marketed by trade names Recombivax and Engerix-B. India is the fourth country in the world after USA, France and Belgium to develop an indigenous hepatitis B vaccine.



**Fig. 10.2 Production of recombinant HB Vaccine**



## 10.2 Gene Therapy

If a person is born with a hereditary disease, can a corrective therapy be given for such disease? Yes, this can be done by a process known as gene therapy. This process involves the transfer of a normal gene into a person's cells that carries one or more mutant alleles. Expression of normal gene in the person results in a functional gene product whose action produces a normal phenotype. Delivery of the normal gene is accomplished by using a vector. The main thrust of gene therapy has been directed at correcting single gene mutations as in cystic fibrosis and haemophilia. At present

most genetic diseases have no effective treatment and so gene therapy could offer hope for many people. There are two strategies involved in gene therapy namely; **Gene augmentation** therapy which involves insertion of DNA into the genome to replace the missing gene product and **Gene inhibition** therapy which involves insertion of the anti sense gene which inhibits the expression of the dominant gene (**Fig. 10.3**).

The two approaches to achieve gene therapy are **somatic** cell and **germ line gene therapy**. Somatic cell therapy involves the insertion of a fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently whereas Germline gene therapy

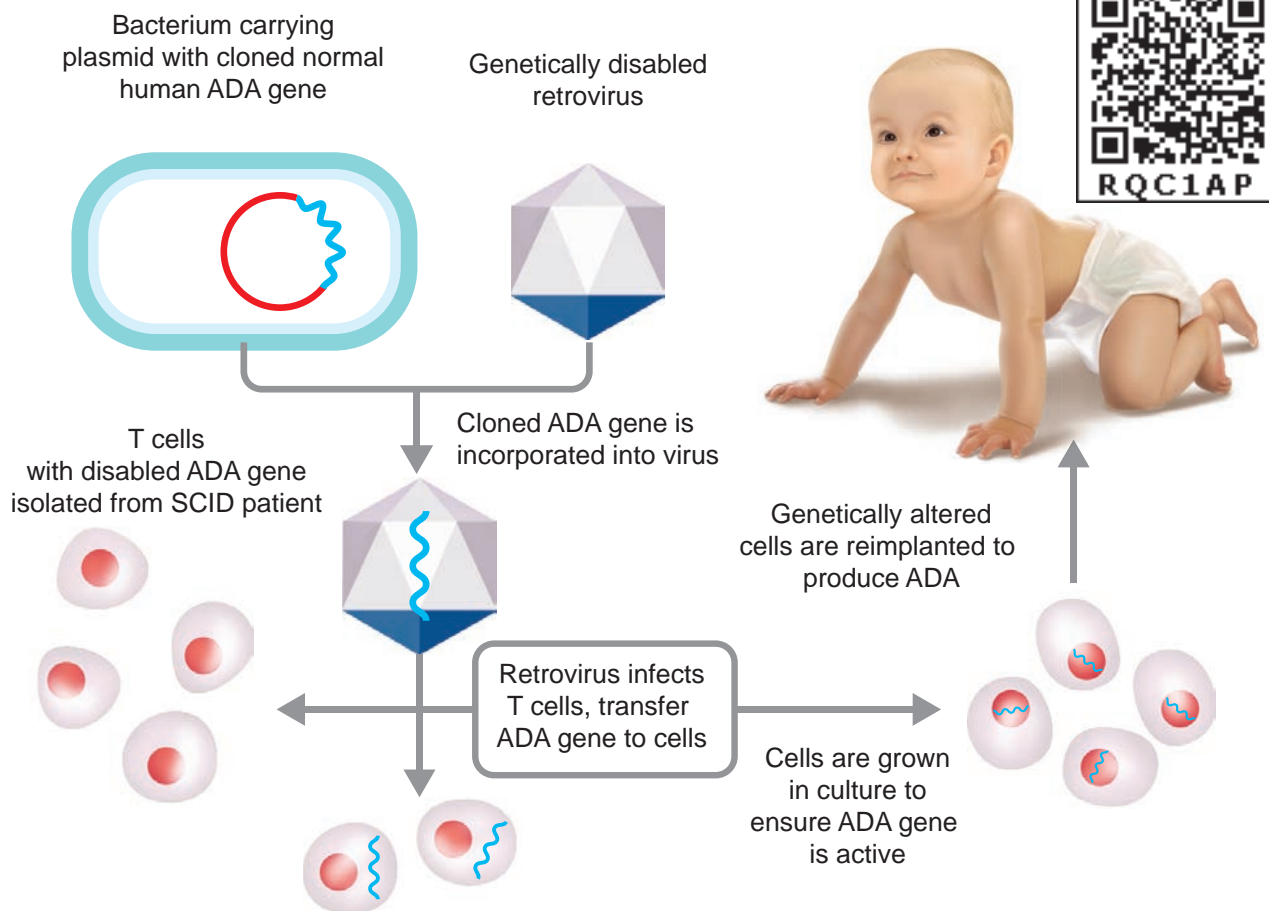
**Table 10.1** Diffentiation between somatic cell gene therapy and germ line gene therapy

SOMATIC CELL GENE THERAPY	GERM LINE GENE THERAPY
Therapeutic genes transferred into the somatic cells.	Therapeutic genes transferred into the germ cells.
Introduction of genes into bone marrow cells, blood cells, skin cells etc.,	Genes introduced into eggs and sperms.
Will not be inherited in later generations.	Heritable and passed on to later generations.

The first clinical gene therapy was given in 1990 by French Anderson to a four year old girl with adenosine deaminase (ADA) deficiency. ADA deficiency or SCID (Severe combined immunodeficiency) is an autosomal recessive metabolic disorder. It is caused by the deletion or dysfunction of the gene coding for ADA enzyme. In these patients the nonfunctioning T-Lymphocytes cannot elicit immune responses against invading pathogens. The right approach for SCID treatment would be to give the patient a functioning ADA which breaks down toxic biological products.

In some children ADA deficiency could be cured by bone marrow transplantation, where defective immune cells could be replaced with healthy immune cells from a donor. In some patients it can be treated by enzyme replacement therapy, in which functional ADA is injected into the patient.

During gene therapy the lymphocytes from the blood of the patient are removed and grown in a nutrient culture medium. A healthy and functional human gene, ADA cDNA encoding this enzyme is introduced into the lymphocytes using a retrovirus. The genetically engineered lymphocytes are subsequently returned to the patient. Since these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. The disease could be cured permanently if the gene for ADA isolated from bone marrow cells are introduced into the cells of the early embryonic stages.



**Fig. 10.3 Process of gene therapy**

involves the introduction of DNA into germ cells which is passed on to the successive generations. Gene therapy involves isolation of a specific gene and making its copies and inserting them into target cells to make the desired proteins. It is absolutely essential for gene therapists to ensure that the gene is harmless to the patient and it is appropriately expressed and that the body's immune system does not react to the foreign proteins produced by the new genes.

### 10.3 Stem Cell Therapy

Stem cells are undifferentiated cells found in most of the multi cellular animals. These cells maintain their undifferentiated state even after undergoing numerous mitotic divisions.

Stem cell research has the potential to revolutionize the future of medicine with the ability to regenerate damaged and diseased organs. Stem cells are capable of self renewal and exhibit 'cellular potency'. Stem cells can

differentiate into all types of cells that are derived from any of the three germ layers ectoderm, endoderm and mesoderm.

In mammals there are two main types of stem cells – embryonic stem cells (ES cells) and adult stem cells. ES cells are pluripotent and can produce the three primary germ layers ectoderm, mesoderm and endoderm. Embryonic stem cells are multipotent stem cells that can differentiate into a number of types of cells (**Fig. 10.4**). ES cells are isolated from the epiblast tissue of the inner cell mass of a blastocyst. When stimulated ES can develop into more than 200 cells types of the adult body. ES cells are immortal i.e., they can proliferate in a sterile culture medium and maintain their undifferentiated state.

Adult stem cells are found in various tissues of children as well as adults. An adult stem cell or somatic stem cell can divide and create another cell similar to it. Most of the adult stem cells are

multipotent and can act as a repair system of the body, replenishing adult tissues. The red bone marrow is a rich source of adult stem cells.

The most important and potential application of human stem cells is the generation of cells and tissues that could be used for cell based therapies. Human stem cells could be used to test new drugs.

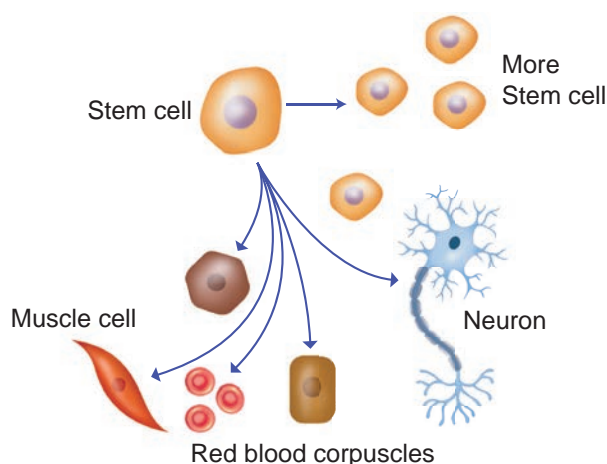
**Totipotency (Toti-total)** is the ability of a single cell to divide and produce all of the differentiated cells in an organism.

**Pluripotency (Pluri-several)** refers to a stem cell that has the potential to differentiate into any of the three germ layers-ectoderm, endoderm and mesoderm.

**Multipotency (multi-Many)** refers to the stem cells that can differentiate into various types of cells that are related. For example blood stem cells can differentiate into lymphocytes, monocytes, neutrophils etc.,

**Oligopotency (Oligo-Few)** refers to stem cells that can differentiate into few cell types. For example lymphoid or myeloid stem cells can differentiate into B and T cells but not RBC.

**Unipotency (Uni- Single)** refers to the ability of the stem cells to differentiate into only one cell type.



**Fig. 10.4 Embryonic stem cells**

### Stem Cell Banks

Stem cell banking is the extraction, processing and storage of stem cells, so that

they may be used for treatment in the future, when required. Amniotic cell bank is a facility that stores stem cells derived from amniotic fluid for future use. Stem cells are stored in banks specifically for use by the individual from whom such cells have been collected and the banking costs are paid. Cord Blood Banking is the extraction of stem cells from the umbilical cord during childbirth. While the umbilical cord and cord blood are the most popular sources of stem cells, the placenta, amniotic sac and amniotic fluid are also rich sources in terms of both quantity and quality.

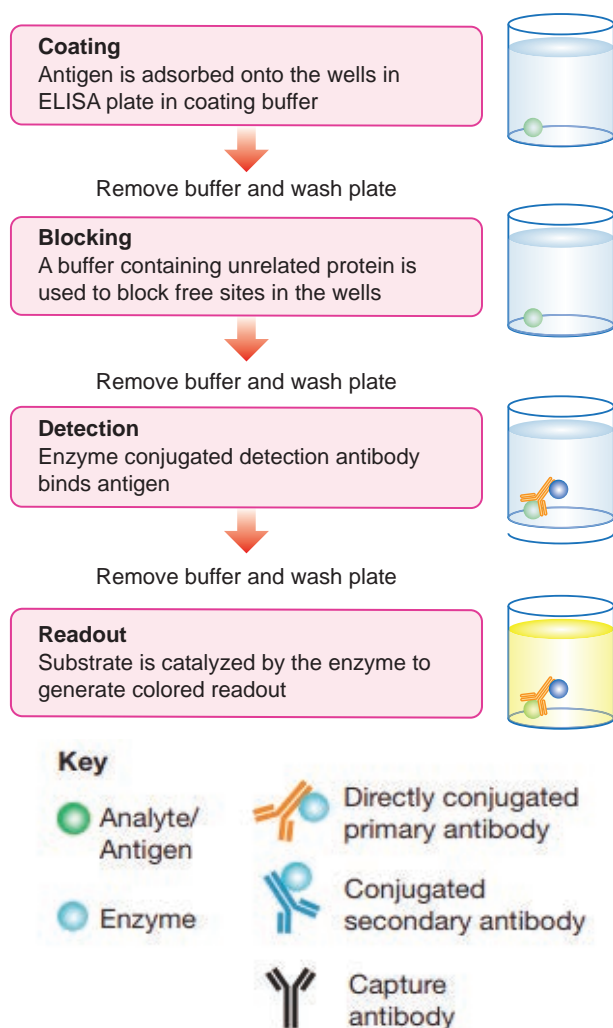
## 10.4 Molecular Diagnostics

Early diagnosis of infectious diseases or inherent genetic defects is essential for appropriate treatment. Early detection of the disease is not possible using conventional diagnostic methods like microscopic examinations, serum analysis and urine analysis. These laboratory techniques are indirect and not always specific. Scientists are continuously searching for specific, sensitive and simple diagnostic techniques for diagnosis of diseases. Recombinant DNA technology, Polymerase Chain Reactions (PCR) and Enzyme Linked Immunosorbent Assay (ELISA) are some of the techniques that are reliable and help in early diagnosis. Presence of pathogens like virus, bacteria, etc., is detected only when the pathogen produces symptoms in the patient. By the time the symptoms appear concentration of pathogen becomes very high in the body. However very low concentration of a bacteria or a virus, even when the symptoms of the disease does not appear, can be detected by amplification of their nucleic acid.

**ELISA [Enzyme Linked Immunosorbent Assay]**

ELISA is a biochemical procedure discovered by **Eva Engvall** and **Peter Perlmanin (1971)** to detect the presence of specific antibodies or antigens in a sample

of serum, urine, etc., It is a very important diagnostic tool to determine if a person is HIV positive or negative. ELISA is a tool for determining serum antibody concentrations (such as the antibodies produced in a person infected by pathogens such as HIV) and also for detecting the presence of specific antigens and hormones such as human chorionic gonadotropins.



**Fig. 10.5 Enzyme Linked Immuno Sorbent Assay**

During diagnosis the sample suspected to contain the antigen is immobilized on the surface of an ELISA plate (Fig. 10.5). The antibody specific to this antigen is added and allowed to react with the immobilized antigen. The anti-antibody is linked to an appropriate enzyme like peroxidase. The unreacted anti-antibody is washed away

and the substrate of the enzyme (hydrogen peroxidase) is added with certain reagents such as 4-chloronaphthol. The activity of the enzyme yields a coloured product indicating the presence of the antigen. The intensity of the colour is directly proportional to the amount of the antigen. ELISA is highly sensitive and can detect antigens in the range of a nanogram.

There are four kinds of ELISA namely, Direct ELISA, Indirect ELISA, sandwich ELISA and competitive ELISA. It is a highly sensitive and specific method used for diagnosis. ELISA possesses the added advantages of not requiring radioisotopes or a radiation counting apparatus.

#### PCR (Polymerase Chain Reaction)

The polymerase chain reaction (PCR) is an *invitro* amplification technique used for synthesising multiple identical copies (billions) of DNA of interest. The technique was developed by **Kary Mullis** (Nobel laureate, 1993) in the year 1983.

Denaturation, renaturation or primer annealing and synthesis or primer extension, are the three steps involved in PCR (Fig. 10.6). The double stranded DNA of interest is denatured to separate into two individual strands by high temperature. This is called **denaturation**. Each strand is allowed to hybridize with a primer (renaturation or primer annealing). The primer template is used to synthesize DNA by using Taq – DNA polymerase.

During denaturation the reaction mixture is heated to 95° C for a short time to denature the target DNA into single strands that will act as a template for DNA synthesis. Annealing is done by rapid cooling of the mixture, allowing the primers to bind to the sequences on each of the two strands flanking the target DNA. During primer extension or synthesis the temperature of the mixture is



increased to 75°C for a sufficient period of time to allow Taq DNA polymerase to extend each primer by copying the single stranded template. At the end of incubation both single template strands will be made partially double stranded. The new strand of each double stranded DNA extends to a variable distance downstream. These steps are repeated again and again to generate multiple forms of the desired DNA. This process is also called DNA amplification.

The PCR technique can also be used for amplifications of RNA in which case it is referred to as reverse transcription PCR (RT-PCR). In this process the RNA molecules (mRNA) must be converted to complementary DNA by the enzyme reverse transcriptase. The cDNA then serves as the template for PCR.

### PCR In Clinical Diagnosis

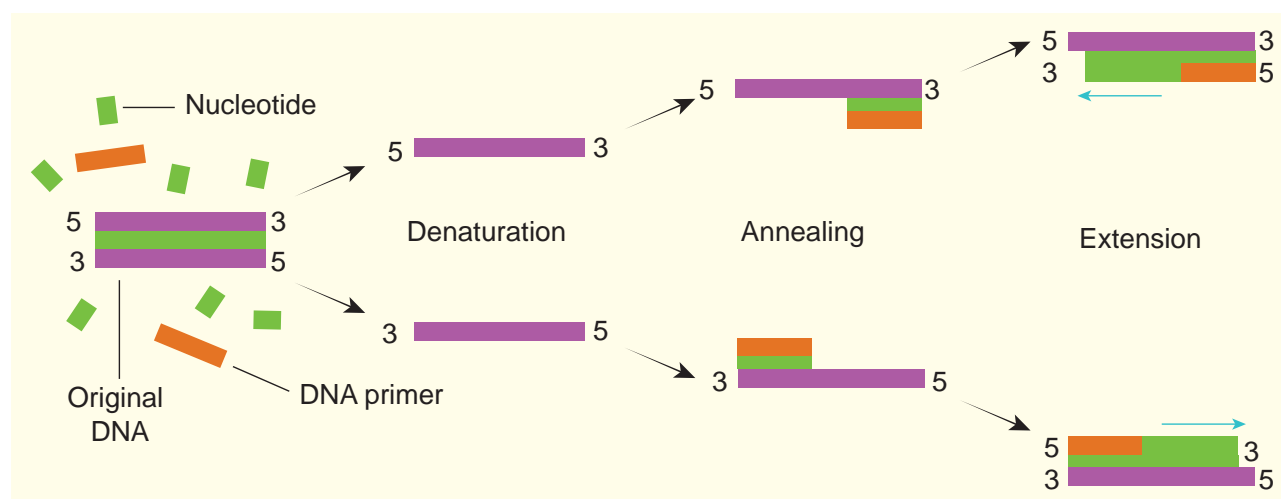
The specificity and sensitivity of PCR is useful for the diagnosis of inherited disorders (genetic diseases), viral diseases, bacterial diseases, etc., The diagnosis and treatment of a particular disease often requires identifying a particular pathogen. Traditional methods of identification involve culturing these organisms from clinical specimens and performing metabolic and other tests to identify them. The concept

behind PCR based diagnosis of infectious diseases is simple – if the pathogen is present in a clinical specimen its DNA will be present. Its DNA has unique sequences that can be detected by PCR, often using the clinical specimen (for example, blood, stool, spinal fluid, or sputum) in the PCR mixture. PCR is also employed in the prenatal diagnosis of inherited diseases by using chorionic villi samples or cells from amniocentesis. Diseases like sickle cell anemia,  $\beta$ -thalassemia and phenylketonuria can be detected by PCR in these samples. cDNA from PCR is a valuable tool for diagnosis and monitoring retroviral infections e.g., Tuberculosis by *Mycobacterium tuberculosis*.

Several virally induced cancers, like cervical cancer caused by Papilloma virus can be detected by PCR. Sex of human beings and live stocks, embryos fertilized *invitro* can be determined by PCR by using primers and DNA probes specific for sex chromosomes. PCR technique is also used to detect sex-linked disorders in fertilized embryos.

### Applications of PCR

The differences in the genomes of two different organisms can be studied by PCR. PCR is very important in the study of evolutions, more specifically phylogenetics. As a technique which can amplify even



**Fig. 10.6 Steps involved in PCR**

minute quantities of DNA from any source, like hair, mummified tissues, bones or any fossilized materials.

PCR technique can also be used in the field of forensic medicine. A single molecule of DNA from blood stains, hair, semen of an individual is adequate for amplification by PCR. The amplified DNA is used to develop DNA fingerprint which is used as an important tool in forensic science. Thus, PCR is very useful for identification of criminals. PCR is also used in amplification of specific DNA segment to be used in gene therapy.

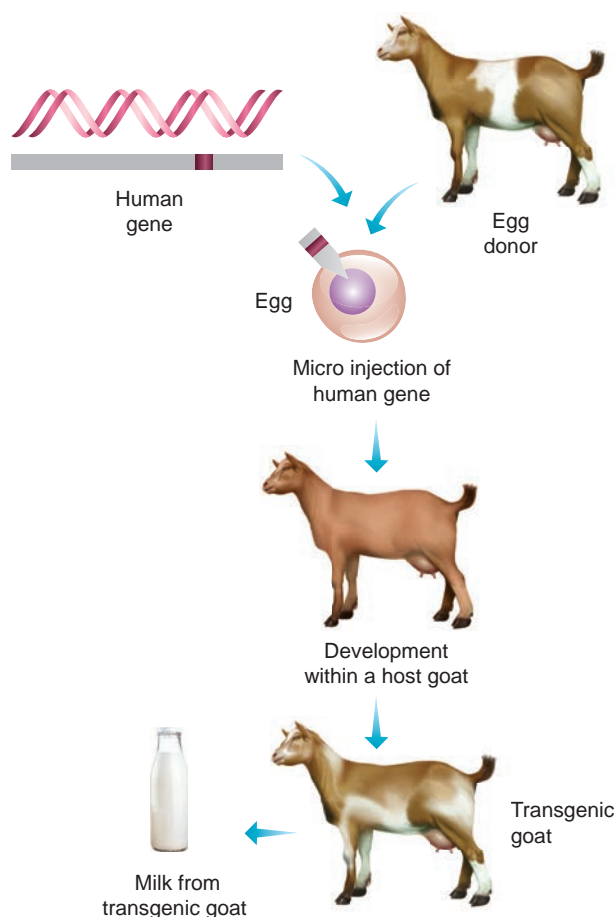
### 10.5 Transgenic Animals

In early days selective breeding methods were carried out to improve the genetic characteristics of live stock and other domestic animals. With the advent of modern biotechnology it is possible to carry out manipulations at the genetic level to get the desired traits in animals. **Transgenesis** is the process of introduction of extra (foreign/exogenous) DNA into the genome of the animals to create and maintain stable heritable characters. The foreign DNA that is introduced is called the transgene and the animals that are produced by DNA manipulations are called **transgenic animals** or the **genetically engineered** or genetically modified organisms.

The various steps involved in the production of transgenic organisms are

- Identification and separation of desired gene.
- Selection of a vector (generally a virus) or direct transmission.
- Combining the desired gene with the vector.
- Introduction of transferred vector into cells, tissues, embryo or mature individual.
- Demonstration of integration and expression of foreign gene in transgenic

tissue or animals. Transgenic animals such as mice, rat, rabbit, pig, cow, goat, sheep and fish have been produced (Fig. 10.7).



**Fig. 10. 7 Production of transgenic animals**

#### Uses Of Transgenesis

- Transgenesis is a powerful tool to study gene expression and developmental processes in higher organisms.
- Transgenesis helps in the improvement of genetic characters in animals. Transgenic animals serve as good models for understanding human diseases which help in the investigation of new treatments for diseases. Transgenic models exist for many human diseases such as cancer, Alzheimer's, cystic fibrosis, rheumatoid arthritis and sickle cell anemia.
- Transgenic animals are used to produce proteins which are important for medical and pharmaceutical applications.



- Transgenic mice are used for testing the safety of vaccines.
- Transgenic animals are used for testing toxicity in animals that carry genes which make them sensitive to toxic substances than non-transgenic animals exposed to toxic substances and their effects are studied.
- Transgenesis is important for improving the quality and quantity of milk, meat, eggs and wool production in addition to testing drug resistance.

### 10.6 Biological products and their uses

A biological product is a substance derived from a living organism and used for the prevention or treatment of disease. These products include antitoxins, bacterial and viral vaccines, blood products and hormone extracts. These products may be produced through biotechnology in a living system, such as a microorganism, plant cell or animal cell, and are often more difficult to characterize than small molecule drugs. Through recombinant DNA technology it is possible to produce these biological products on demand. There are many types of biological products approved for use -they are, therapeutic proteins, monoclonal antibodies and vaccines. Health care and pharmaceutical industries have been revolutionised by biotechnological proteins. Hormones and antibodies are produced commercially, primarily for the medical industry. Recombinant hormones like Insulin, Human growth hormone, Recombinant vaccines and recombinant proteins like human alpha lactalbumin are available today.

Animals are used as bioreactors to produce desirable proteins. Antibodies are substances that react against the disease causing antigens and these can be produced using transgenic animals as bioreactors. Monoclonal antibodies, which are used to treat cancer, heart disease

and transplant rejection are produced by this technology. Natural protein adhesives are non toxic, biodegradable and rarely trigger an immune response, hence could be used to reattach tendons and tissues, fill cavities in teeth, and repair broken bones.

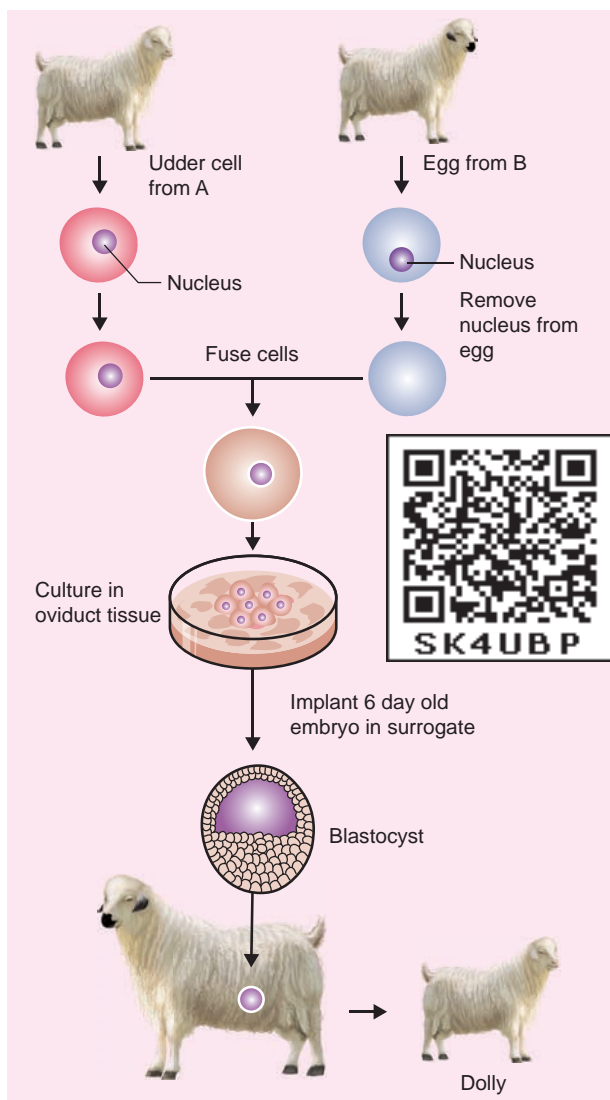
### 10.7 Animal Cloning

Cloning is the process of producing genetically identical individuals of an organism either naturally or artificially. In nature many organisms produce clones through asexual reproduction.

Cloning in biotechnology refers to the process of creating copies of organisms or copies of cells or DNA fragments (molecular cloning).

Dolly was the first mammal (Sheep) clone developed by Ian Wilmut and Campbell in 1997. Dolly, the transgenic clone was developed by the nuclear transfer technique and the phenomenon of totipotency. **Totipotency** refers to the potential of a cell to develop different cells, tissues, organs and finally an organism.

The mammary gland udder cells (somatic cells) from a donor sheep (ewe) were isolated and subjected to starvation for 5 days. The udder cells could not undergo normal growth cycle, entered a dormant stage and became totipotent. An ovum (egg cell) was taken from another sheep (ewe) and its nucleus was removed to form an enucleated ovum. The dormant mammary gland cell/udder cell and the enucleated ovum were fused. The outer membrane of the mammary cell was ruptured allowing the ovum to envelope the nucleus. The fused cell was implanted into another ewe which served as a surrogate mother. Five months later dolly was born. Dolly was the first animal to be cloned from a differentiated somatic cell taken from an adult animal without the process of fertilization (**Fig. 10.8**).



**Fig. 10. 8 Cloning of dolly**

### Advantages and Disadvantages Of Cloning Animals

- Offers benefits for clinical trials and medical research. It can help in the production of proteins and drugs in the field of medicine.
- Aids stem cell research.
- Animal cloning could help to save endangered species.
- Animal and human activists see it as a threat to biodiversity saying that this alters evolution which will have an impact on populations and the ecosystem.
- The process is tedious and very expensive.
- It can cause animals to suffer.

- Reports show that animal surrogates were manifesting adverse outcomes and cloned animals were affected with disease and have high mortality rate.
- It might compromise human health through consumption of cloned animal meat.
- Cloned animals age faster than normal animals and are less healthy than the parent organism as discovered in Dolly.
- Cloning can lead to occurrence of genetic disorders in animals.
- More than 90% of cloning attempts fail to produce a viable offspring.



Ian Wilmut and Campbell removed 277 cells from the udder of an adult sheep and fused those cells with 277 unfertilised egg cells from which the nuclear material was removed. After culturing the resulting embryos for 6 days, they implanted 29 embryos into the surrogate mother's womb and only one Dolly was produced.

A gene 'knock out' is a genetically engineered organism that carries one or more genes in its chromosomes that have been made inoperative.

### 10.8 Ethical Issues

Biotechnology has given to the society cheap drugs, better fruits and vegetables, pest resistant crops, indigenous cure to diseases and lot of controversy. This is mainly because the major part of the modern biotechnology deals with genetic manipulations. People fear that these genetic manipulations may lead to unknown consequences. The major apprehension of recombinant DNA technology is that unique microorganisms either inadvertently or deliberately for the purpose of war may



be developed that could cause epidemics or environmental catastrophies. Although many are concerned about the possible risk of genetic engineering, the risks are in fact slight and the potential benefits are substantial.

## 10.9 Regulations in Biotechnology

Regulations apply to the production, sale and use of biotech products and genetically modified organisms. GMOs are carefully tested and documented before the products are available. GMOs should be labelled and used according to instructions. These regulations are designed to protect the people, living organisms and the environment. The Biotechnology Regulatory Authority of India (BRAI) is a proposed regulatory body in India for uses of biotechnology products including GMOs. The Genetic Engineering Approval Committee (GEAC), a body under the Ministry of Environment, forests and climate change (India) is responsible for approval of genetically engineered products in India. If the bill is passed the responsibility will be taken over by the Environmental Appraisal Panel, a subdivision of the BRAI. The bill also proposes setting up an inter ministerial governing body to oversee the performance of BRAI and a National Biotechnology Advisory Council of stakeholders to provide feedback on the use of, import and manufacture of biotechnology products and organisms in the society. The regulatory body is an autonomous and statutory agency to regulate the research, transport, import and manufacture of biotechnology products and organisms.

GEAC is assisted by the State Biotechnology Co-ordination Committee (SBCC) and District Level committee (DLC). The most important committees are The Institutional Biosafety Committee (IBSC), responsible for the local implementation of

guidelines; Review Committee on Genetic Manipulation (RCGM) is responsible for issuing permits and the GEAC is responsible for monitoring the large scale and commercial use of transgenic materials.

The biotechnology industry is governed by different enactments depending on their relevance / applicability on a case to case basis. "Recombinant DNA safety guidelines, 1990" were released by the Department of Biotechnology (DBT) which cover areas of research involving genetically engineered organisms and these guidelines were further revised in 1994.

RCGM under the DBT comprises representatives of DBT, Indian Council for Medical Research, Indian Council for Agricultural research and Council for Scientific and Industrial Research.

Industrial licensing under the Industrial (Development and Regulation) Act, 1951 is compulsory for bulk drugs produced by the use of recombinant DNA technology.

Being a signatory to the Trade Related Intellectual Property Rights (TRIPS) Agreement of WTO, India has amended its legislations pertaining to intellectual property through various legislations including Patents (Amendment) Act, 1999.

1. The Recombinant DNA Advisory Committee (RDAC)	→ <b>Advisory</b>
2. Institutional Biosafety Committee (IBSC)	} <b>Approval</b>
3. Review Committee on Genetic Manipulation (RCGM)	
4. Genetic Engineering Approval Committee (GEAC)	
5. State Biotechnology Coordination Committee (SBCC)	} <b>Monitoring</b>
6. District Level Committee (DLC)	

**Biopiracy** can be defined as “the use of bioresources by multinational companies and other organisations without proper authorization from the countries and the people concerned without compensatory payment”.

**Bioethics** is the study of the ethical issues emerging from the advances in Biology and medicine. It is also a moral discernment as it relates to the medical policy and practice.

### 10.10 Possible threats of Genetically Modified Organisms

Genetically Modified Organisms (GMOs) also called Genetically Engineered organisms (GEOs) are created to play a role in agriculture, forestry, aquaculture, bioremediation and environmental management in developed and developing countries. However, deliberate or inadvertent release of GMOs into the environment could have negative ecological effects under certain circumstances.

### The possible risks of GMOs

Creating new or more vigorous pests and pathogens. Worsening the effects of existing pests through hybridization with related transgenic organisms (**Table 10.2**).

- Harming non-target species such as soil organisms, non-pest insects, birds and other animals.
- Disrupting biotic communities including agro ecosystems.
- Irreparable loss or changes in species diversity or genetic diversity within species.
- Creating risks for human health.

The release of GMOs into the environment could also have far reaching consequences. This is because the living GMOs proliferate, persist, disperse and sometimes may transfer their DNA into other organisms. GEOs could also displace the existing organism and create new species which may cause severe environmental damage. Due to these risks the regulatory authorities are very careful in permitting the field trials of GMOs into the environment.

**Table 10.2. Risks of GMOs**

Environmental	Health	Agricultural
Toxins in pest-resistant GMOs could negatively impact non-target organisms and harm ecosystems.	Proteins transcribed and translated from transferred genes could cause allergic reactions in humans or other animals – currently GM foods are not properly labelled.	GMOs with pest toxins could increase evolution of resistance in certain pest populations.
Cross-species pollination could spread herbicide resistance genes and create 'super-weeds'.	Antibiotic resistance genes used as markers during gene transfer could spread to pathogenic bacteria.	Big biotech companies hold monopolistic legal rights (patents) over GM seeds.
Biodiversity could be negatively affected by destruction of pests, weeds, and even competing plants.	Transferred genes could mutate and cause unexpected risks.	GMOs do present two major agricultural problems in the forms of pesticide- and herbicide-resistance.

## 10.11 Biosafety Guidelines

Due to the growing concerns arising from Genetically Modified Organisms (GMOs) throughout the globe the WHO has built an informal working group on biosafety in 1991. This group prepared the 'voluntary code for the release of organisms into the environment'. ICGEB (International Centre for Genetic Engineering and Biotechnology) has played a significant role in issues related to biosafety and the environmentally sustainable use of biotechnology. The main 'topic of concern' related to the release of GMO's are risks for human health, environment, and agriculture which is found on the website of ICGEB.

In India, DBT has evolved 'rDNA safety guidelines' to exercise powers conferred through the Environmental Protection Act 1986 for the manufacture, use, import, export and storage of hazardous micro organisms and genetically engineered organisms, cells etc., These guidelines are implemented and monitored by the Institutional Biosafety Committees (IBSCs), the Review Committee on Genetic Manipulation (RCGM) and the Genetic Engineering Approval Committee (GEAC) of the Ministry of Environment and Forest.

### Intellectual Property Rights (IPR) and Protection (IPP)

The physical objects like household goods or land or properties of a person and the ownership and rights on these properties is protected by certain laws operating in the country. This type of physical property is tangible; but the transformed microorganisms, plants, animals and technologies for the production of commercial products are exclusively the property of the intellectuals. The discoverer or inventor has complete rights on his property or invention. The rights of intellectuals are protected by laws framed by a country. The intellectual property is an intangible asset. Legal rights or patents provide an inventor only a temporary monopoly on the use of an invention,

in return for disclosing the knowledge to the others who may use the knowledge to develop further inventions and innovations.

The laws are formulated from time to time at national and international levels. Development of new crop varieties is also an intellectual property right. It is protected by 'plant breeders rights' (PBRs). PBRs recognize the fact that farmers and rural communities have contributed to the creation, conservation, exchange and knowledge of genetic and species utilization of genetic diversity. IPR and IPP are granted by the Government to plant breeders for producing a specific plant variety that is new and never existed before.

IPR is protected by different ways like patents, copyrights and trade marks.

### Patents

The science of biotechnology involves the production of enormous number of commercial products of economic importance. The inventions include biotechnology products and processes. The products include living entities like micro organisms, animals, plants, cell lines, cell organelles, plasmids and genes and naturally occurring products like primary and secondary metabolites produced by living systems e.g. alcohol, antibiotics.

Thomas Alva Edison was the only scientist in the world with more than 1000 patents to his credit.

The biotechnological processes involve isolation, purification, cultivation, bioconversion of novel, innovative, simple and cost effective processes, and creation of biotechnological products.

A patent is a Government issued document that allows the person for an exclusive right to manufacture, use or sell an invention for a defined period (usually 20 years). It is a legal document safeguarding the rights and privileges of an inventor / invention. The purpose of

patenting in biotechnology ensures fair financial returns for those who have invested finances, ideas, time and hard work for an invention.

The following criteria must be satisfied for patenting :

- The invention must be novel and useful;
- The product must be inventive and reproducible;
- The patent application should provide the full description of the invention and the invention must be patentable.

**DO YOU KNOW?** The first living organism that was patented was a genetically engineered species of bacteria - *Pseudomonas putida* in 1980 which was genetically engineered by Ananda Mohan Chakrabarty in 1971.

### General agreement of tariffs and trade (GATT) and trade related IPRs (TRIPs)

GATT was framed in 1948 by developed countries to settle dispute, among the countries regarding share of world trade. The benefits of GATT was enjoyed only by developed countries. In 1988 US congress enacted a law 'the omnibus trade and competitiveness act' (OTCA) which gave powers to US to investigate the laws related to trade.

### Geographical indication (GI)

A geographical indication is a name or sign used on products which correspond to a specific geographical origin and possess qualities or a reputation that are due to that origin. Geographical indications are typically used for agricultural products, food products, handicrafts and industrial products. Darjeeling tea was the first GI tagged product in India in 2004-05. In Tamilnadu, Kancheepuram silk, Coimbatore wetgrinder, Thanjavur paintings, Madurai Malli, Thirunelveli Halwa and Temple jewellery of Nagercoil are GI tagged.

### Copyright

The protection of authorships of published work comes under copyrights of IPRs. Copyright protection is given for form of expressions of ideas. For example the authors, editors, publishers or both the publisher/ editor of a book have copyrights. The content of the book cannot be reproduced or reprinted without written permission from copyright holders. Patents and trade secrets provide protection for the basic knowhow but copyright protects the expressed materials in printed, video recorded or taped forms. In the field of biotechnology the data base of DNA sequences or any published forms, photomicrographs, etc., are subject to copyright.

### Trade marks

Any specific symbol or words to identify a particular product or process of a company constitute trademark. This enables the public to distinguish between a trader's goods from similar goods of other traders

Biotechnology as an independent discipline has drawn world wide attention from the Governments and the corporate world because of its limitless applications. It is looked upon as a panacea for treating diseases and genetic disorders. The global demand of the biotechnological products is on the increase. It is the science for the future with solutions to many of the problems related to health, agriculture, environment and industries.

### Summary

Biotechnology is defined as "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use". In 1919, Hungarian agricultural engineer Karl Ereky coined the term Biotechnology. Biotechnology includes two major technologies, Genetic engineering and Chemical engineering.

Biotechnology has application in four major industrial areas, including health care (medical) agriculture, industrial and environment.





Biotechnology techniques are used in the field of medicine for diagnosis, prevention and treatment of different diseases. Production of recombinant hormones, recombinant clotting factor VIII and recombinant interferons have helped in the treatment of diseases. Recombinant vaccines have been used to prevent various diseases. The recombinant vaccines are of three types- subunit recombinant vaccines, attenuated recombinant vaccines and gene recombinant vaccines.

Genetic defects could be corrected by a process called Gene therapy. It is of two types somatic cell gene therapy and germline gene therapy.

Stem cells are undifferentiated cells found in multicellular organisms. These cells are of two types -Embryonic stem cells and adult stem cells. Stem cells have the ability to regenerate damaged and diseased organs. Recombinant DNA technology, Polymerase chain reaction and Enzyme Linked Immunosorbent Assay are techniques that are reliable and help in early diagnosis.

Transgenesis is the process of introduction of a foreign gene into the genome of animals to create and maintain stable heritable characters.

A biological product is a substance derived from a living organism and used for the prevention or treatment of diseases.

Cloning is the process of producing genetically identical individuals of an organism either naturally or artificially.

Advances in Biotechnology and their applications are most frequently associated with controversies, ethical issues and concerns. Statutory bodies are constituted to monitor and approve the biotechnological processes and products.

Genetically modified organisms can play a positive role in sustainable agriculture, forestry, aquaculture, bioremediation and environmental management in developed and developing countries. Biosafety guidelines have been formulated by many countries for DNA recombinant manipulations.

## Evaluation

1. The first clinical gene therapy was done for the treatment of
  - a) AIDS
  - b) Cancer
  - c) Cystic fibrosis
  - d) SCID**
2. Dolly, the sheep was obtained by a technique known as
  - a) Cloning by gene transfer
  - b) Cloning without the help of gametes
  - c) Cloning by tissue culture of somatic cells
  - d) Cloning by nuclear transfer**
3. The genetic defect adenosine deaminase deficiency may be cured permanently by
  - a) Enzyme replacement therapy
  - b) Periodic infusion of genetically engineered lymphocytes having ADA cDNA
  - c) Administering adenosine deaminase activators
  - d) Introducing bone marrow cells producing ADA into embryo at an early stage of development**
4. GEAC stands for
  - a) Genome Engineering Action Committee
  - b) Ground Environment Action Committee
  - c) Genetic Engineering Approval Committee**
  - d) Genetic and Environment Approval Committee
5. How many amino acids are arranged in the two chains of Insulin?
  - a) Chain A has 12 and Chain B has 13
  - b) Chain A has 21 and Chain B has 30 amino acids**
  - c) Chain A has 20 and chain B has 30 amino acids
  - d) Chain A has 12 and chain B has 20 amino acids.
6. PCR proceeds in three distinct steps governed by temperature, they are in order of
  - a) Denaturation, Annealing, Synthesis**
  - b) Synthesis, Annealing, Denaturation
  - c) Annealing, Synthesis, Denaturation
  - d) Denaturation, Synthesis, Annealing





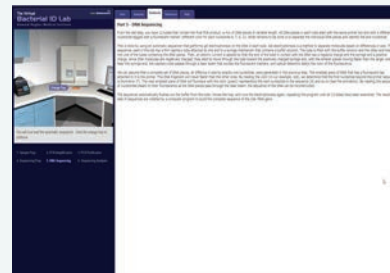
7. Which one of the following statements is true regarding DNA polymerase used in PCR?
  - a) It is used to ligate introduced DNA in recipient cells
  - b) It serves as a selectable marker
  - c) It is isolated from a Virus
  - d) It remains active at a high temperature**
8. ELISA is mainly used for
  - a) Detection of mutations
  - b) Detection of pathogens**
  - c) Selecting animals having desired traits
  - d) Selecting plants having desired traits
9. Transgenic animals are those which have
  - a) Foreign DNA in some of their cells
  - b) Foreign DNA in all their cells**
  - c) Foreign RNA in some of their cells
  - d) Foreign RNA in all their cells
10. Vaccines that use components of a pathogenic organism rather than the whole organism are called
  - a) Subunit recombinant vaccines**
  - b) attenuated recombinant vaccines
  - c) DNA vaccines
  - d) conventional vaccines
11. Mention the number of primers required in each cycle of PCR. Write the role of primers and DNA polymerase in PCR. Name the source organism of the DNA polymerase used in PCR.
12. How is the amplification of a gene sample of interest carried out using PCR?
13. What is genetically engineered Insulin?
14. Explain how “Rosie” is different from a normal cow.
15. How was Insulin obtained before the advent of rDNA technology? What were the problems encountered?
16. ELISA is a technique based on the principles of antigen-antibody reactions. Can this technique be used in the molecular diagnosis of a genetic disorder such as Phenylketonuria?
17. Gene therapy is an attempt to correct a Genetic defect by providing a normal gene into the individual. By this the function can be restored. An alternate method would be to provide gene product known as enzyme replacement therapy, which would also restore the function. Which in your opinion is a better option? Give reasons for your answer.
18. What are transgenic animals? Give examples.
19. If a person thinks he is infected with HIV, due to unprotected sex, and goes for a blood test. Do you think a test such as ELISA will help? If so why? If not, why?
20. Explain how ADA deficiency can be corrected?
21. What are DNA vaccines?
22. Differentiate between Somatic cell gene therapy and germline gene therapy.
23. What are stem cells? Explain its role in the field of medicine.
24. What are the possible risks of GMOs?
25. One of the applications of biotechnology is ‘gene therapy’ to treat a person born with a hereditary disease.
  - i) What does “gene therapy” mean?
  - ii) Name the hereditary disease for which the first clinical gene therapy was used.
  - iii) Mention the steps involved in gene therapy to treat this disease.
26. PCR is a useful tool for early diagnosis of an Infectious disease. Elaborate.
27. What are recombinant vaccines?. Explain the types.
28. Explain why cloning of Dolly, the sheep was such a major scientific breakthrough?
29. Mention the advantages and disadvantages of cloning.
30. Explain how recombinant Insulin can be produced.



## ICT CORNER

### *Applications of biotechnology Science for a better life*

A Transgenic fly. Come let us create and use



#### Procedure :

- Step -1:** Type the **URL** or scan the **QR** code to open the activity page and click “Click to enter the lab” to begin.
- Step -2:** Click wherever the cursor symbol becomes as “**Hand**” symbol as that would lead for the next step.
- Step -3** Follow the instructions for all the six steps “**Prepare DNA** to Examine Light Output”.
- Step – 4 :** Explore the “**Use Transgenic flies**” a dialog box option found below the lab area to know uses.



Step 1



Step 2



Step 3



Step 4

Applications of biotechnology Science for a better life URL:

[http://media.hhmi.org/biointeractive/vlabs/transgenic\\_fly/index.html?\\_ga=2.234689054.1812412497.1546851037-264360672.1545143882](http://media.hhmi.org/biointeractive/vlabs/transgenic_fly/index.html?_ga=2.234689054.1812412497.1546851037-264360672.1545143882)

\*Pictures are indicative only

\*Allow flash player



B267\_12\_ZOO\_EM