UNIT - IV

9 CHAPTER

Applications of Biotechnology

"Our world is built on biology"

Chapter outline

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.*

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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 20th 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.

9.1 Applications in Medicine

9.1.1 Recombinant Human Insulin

The Human insulin is synthesized by the β cells of Islets of Langerhans in the pancreas. It is formed of 51 aminoacids 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. 9.1)**.

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.

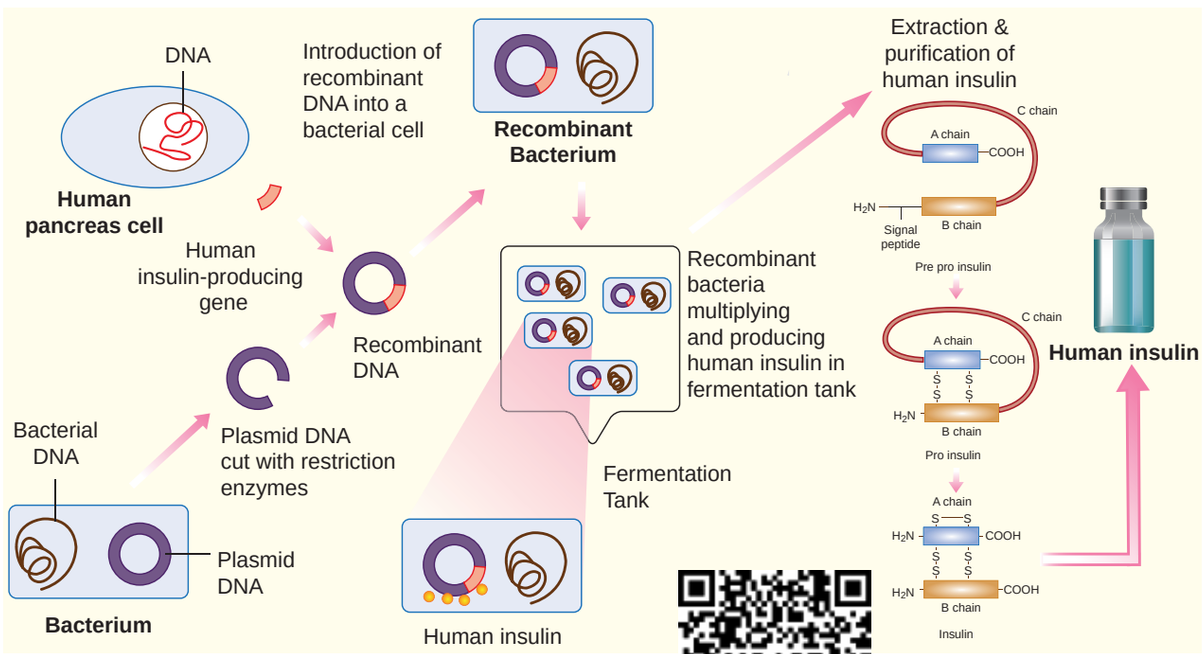

Fig. 9.1 Human Insulin Production

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.

9.1.2 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 α, β and γ 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.

9.1.3 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 is a subunit vaccine. It is produced by combining Hb (Hepatitis B Virus) antigen producing gene in a plasmid DNA obtained from a bacteria. The resultant recombinant DNA is cloned in the yeast, *Saccharomyces cerevisiae* **(Fig. 10.2)**.

9.2 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. 9.3)**.

The two approaches to achieve gene therapy are **somatic** cell and germ **line gene therapy**.

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.

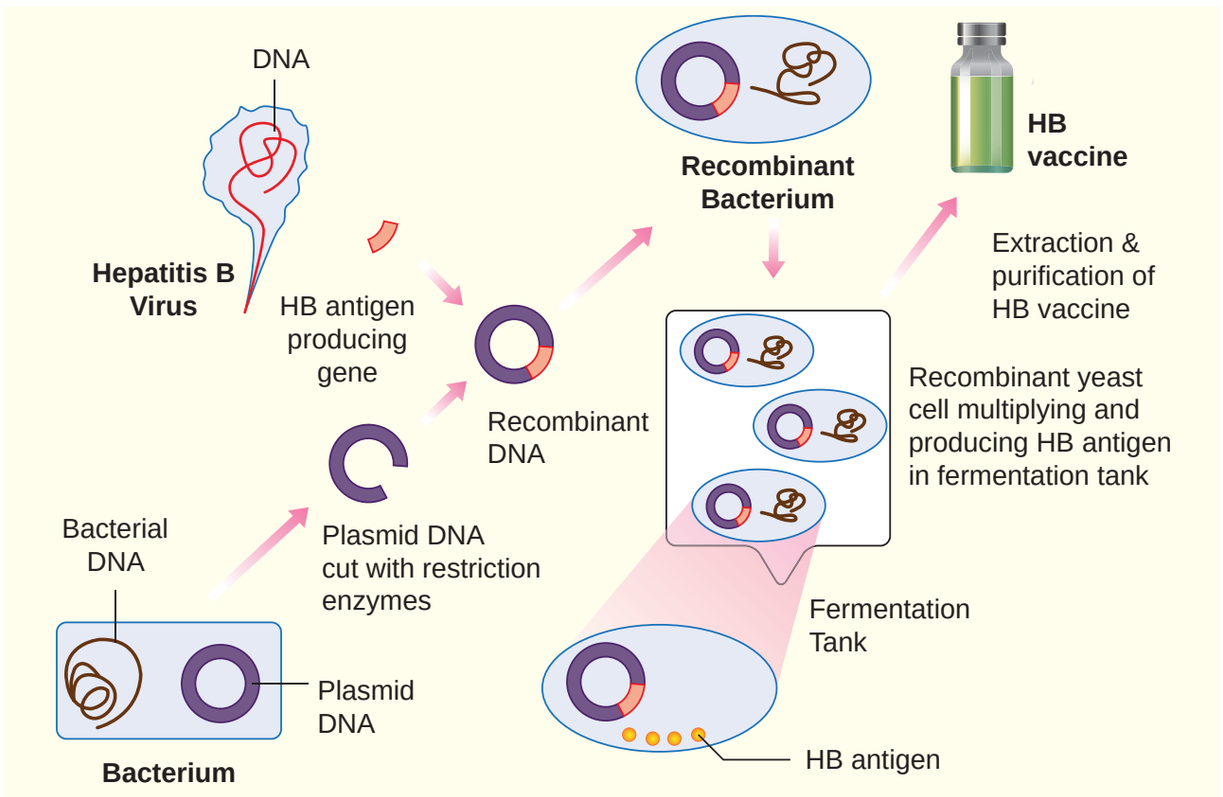

Fig. 9.2 Production of recombinant HB Vaccine

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 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.

9.3 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.

Table 9.1 differentiation 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.

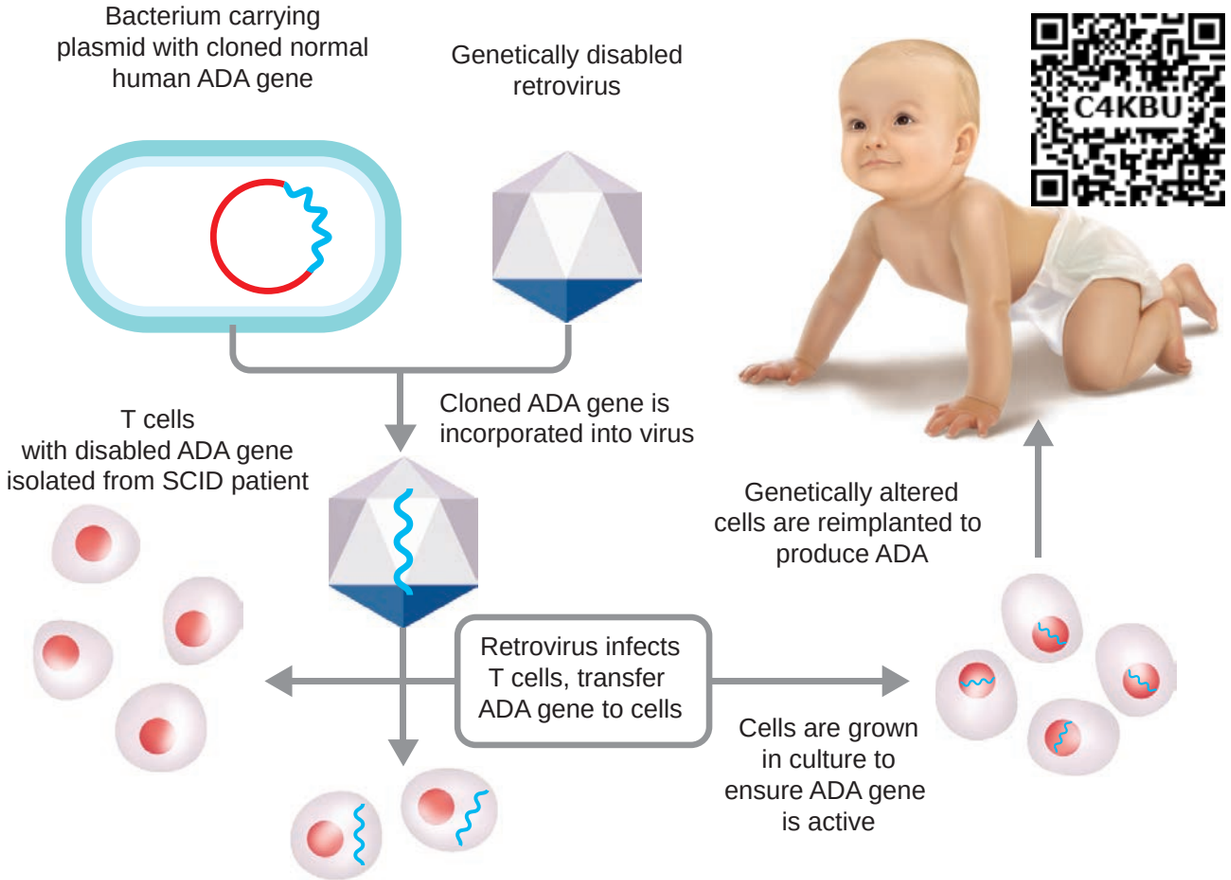

Fig. 9.3 Process of gene therapy

In mammals there are two main types of stem cells – Embryonic Stem Cells (ES Cells) and adult stem cells. ES cells are both pluripotent (can produce the three primary germ layers ectoderm, mesoderm and endoderm) and multipotent (can differentiate into a number of types of cells **(Fig.9.4)**). ES Cells are isolated from the epiblast tissue of the inner cell mass of a blastocyst. When stimulated, ES Cells 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.

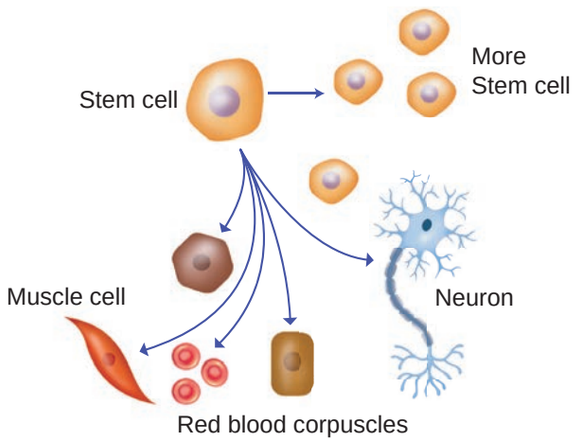

Fig. 9.4 Embryonic stem cells

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.

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.

9.4 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 Perlmann (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.

During diagnosis the sample suspected to contain the antigen is immobilized on the surface of an ELISA plate **(Fig. 9.5)**. 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.

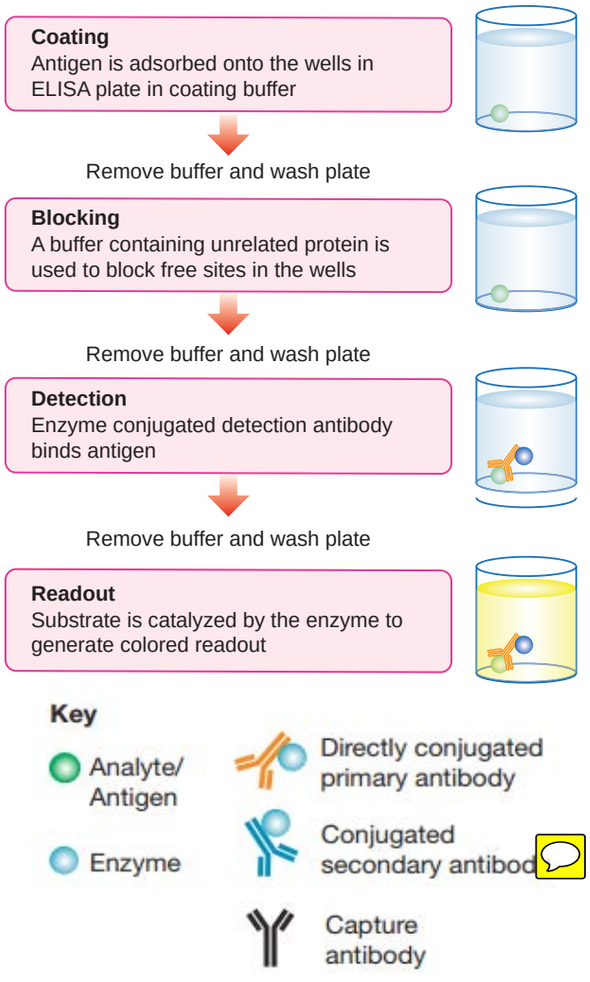

Fig. 9.5 Enzyme Linked Immuno Sorbent Assay

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. 9.6)**. 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 (isolated from the bacterium Thermus aquaticus).

During denaturation the reaction mixture is heated to 95^0 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 75^0C 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.

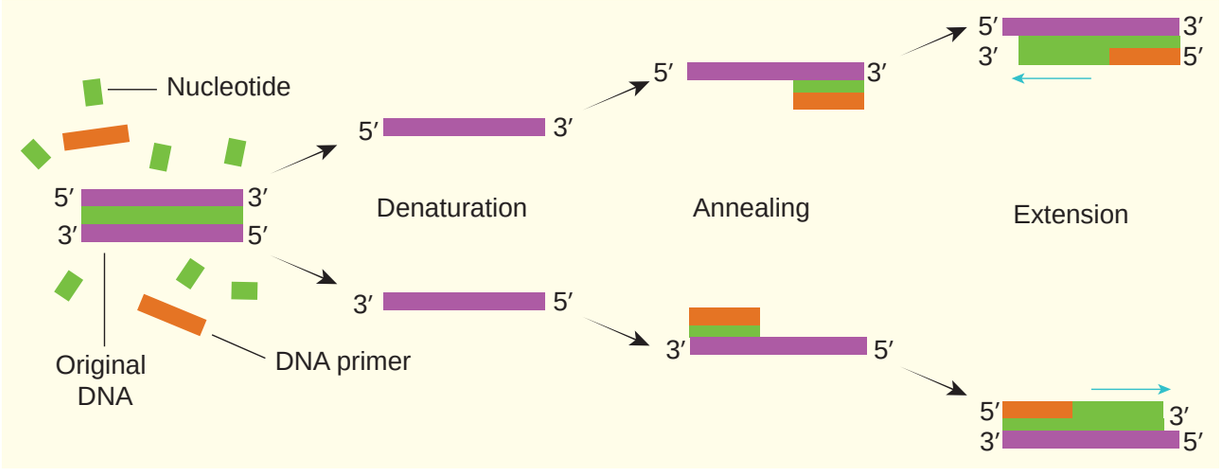

Fig. 9.6 Steps involved in PCR

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, β-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., Corona Virus (SARS-CoV-2).

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 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.

9.5 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.

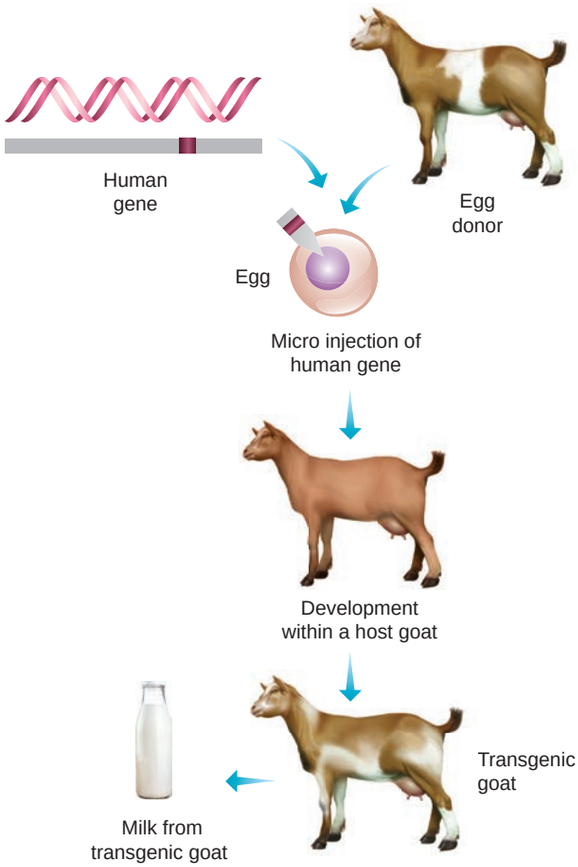

Fig. 9. 7 Production of transgenic animals

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. 9.7)**.

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.

9.6 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.

9.7 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.

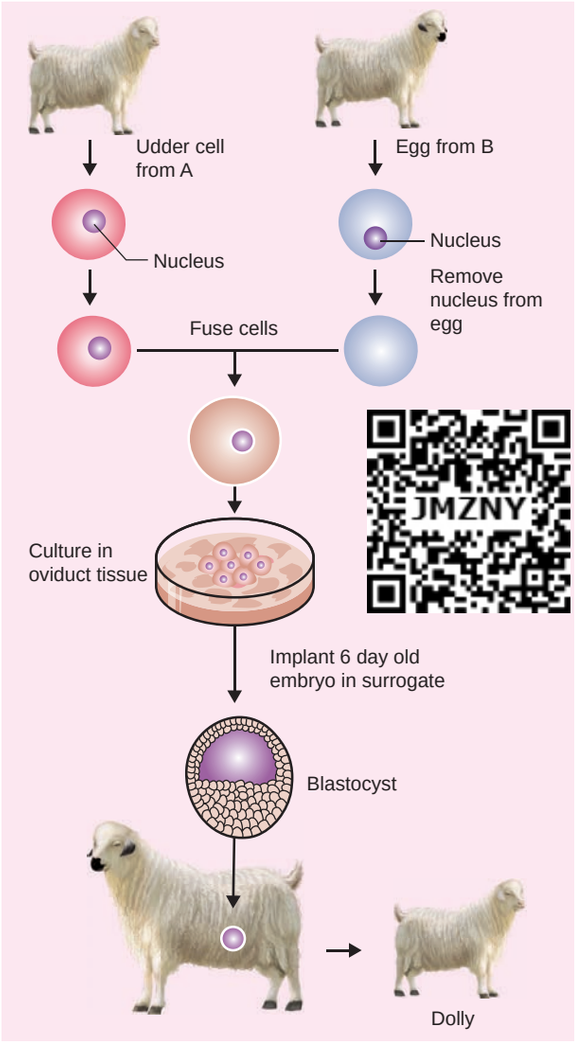

Fig. 9. 8 Cloning of dolly

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. 9.8).**

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.

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.

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

9.8 Ethical Issues

Biotechnology has given to the society cheap drugs, better friuts and vegetables, pest resistant crops, indigenious 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.

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, 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.

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. 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.
5. 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
6. 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
7. 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
8. 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
9. 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
10. 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.

11. How is the amplification of a gene sample of interest carried out using PCR?
12. What is genetically engineered Insulin?
13. Explain how "Rosie" is different from a normal cow.
14. How was Insulin obtained before the advent of rDNA technology? What were the problems encountered?
15. 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?
16. 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.
17. What are transgenic animals? Give examples.
18. 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?
19. Explain how ADA deficiency can be corrected?
20. What are DNA vaccines?
21. Differentiate between Somatic cell gene therapy and germline gene therapy.
22. What are stem cells? Explain its role in the field of medicine.
23. 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.
24. PCR is a useful tool for early diagnosis of an Infectious disease. Elaborate.
25. What are recombinant vaccines?. Explain the types.
26. Explain why cloning of Dolly, the sheep was such a major scientific breakthrough?
27. Mention the advantages and disadvantages of cloning.
28. Explain how recombinant Insulin can be produced.