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## 9. Applications of biotechnology

### Introduction

- Biotechnology is the science of applied **biological process**.
- The term biotechnology was coined by **Karl Ereky**, a Hungarian Engineer in **1919**.
- He was **father of biotechnology**
- Biotechnology has been extended to include any process in which organisms, tissues, cells, organelles or isolated molecules such as enzymes are used to convert biological or other raw materials to products of greater value.
- Biotechnology simply means **the scientific art of using living organism to make desired goods** (character).

### Chapter outline

- 9.1 Applications in Medicine
- 9.2 Gene therapy
- 9.3 Stem Cell Therapy
- 9.4 Molecular Diagnosis
- 9.5 Transgenic Animals
- 9.6 Biological products and their uses
- 9.7 Animal cloning
- 9.8 Ethical issues

### 9.1 Applications in Medicine

- 9.1.1 Recombinant Human Insulin
- 9.1.2 Human Growth Hormone (hGH)
- 9.1.3 Human Blood-Clotting Factor VIII
- 9.1.4 Interferons
- 9.1.5 Recombinant Vaccines

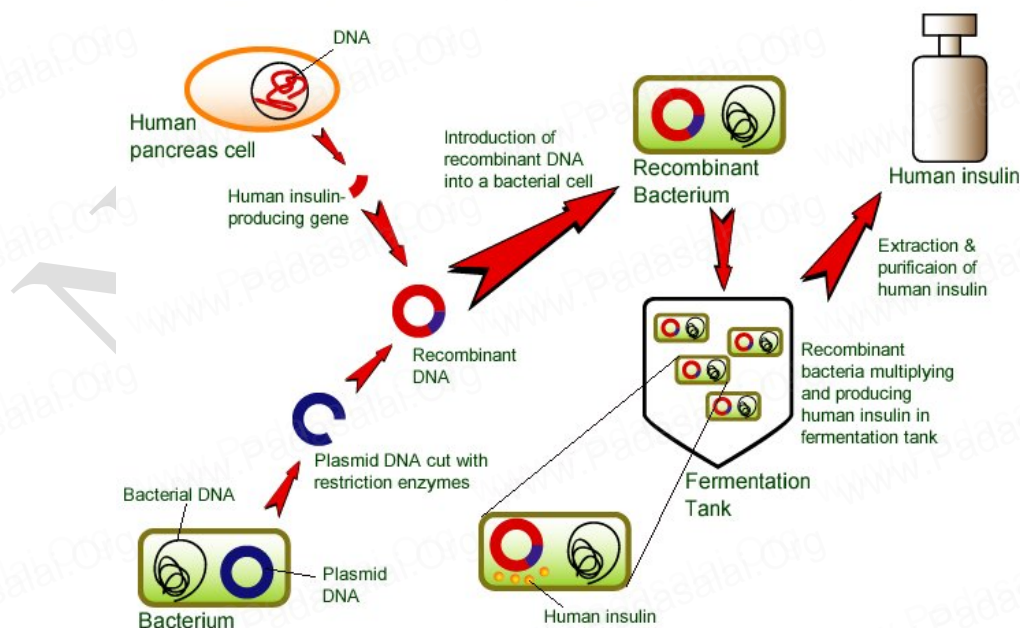
#### **9.1.1 Recombinant Human Insulin**

- ✧ The Human insulin is synthesized by the **β cells of Islets of Langerhans in the pancreas**.
- ✧ It is formed of 51 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.

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- ✧ Both A and B chains are attached together by disulphide bonds.
- ✧ Insulin controls the **levels of glucose** in blood.
- ✧ 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.
- ✧ 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*.
- ✧ Insulin was the **first ever pharmaceutical product** of recombinant DNA technology administered to humans.

### Human Insulin Production

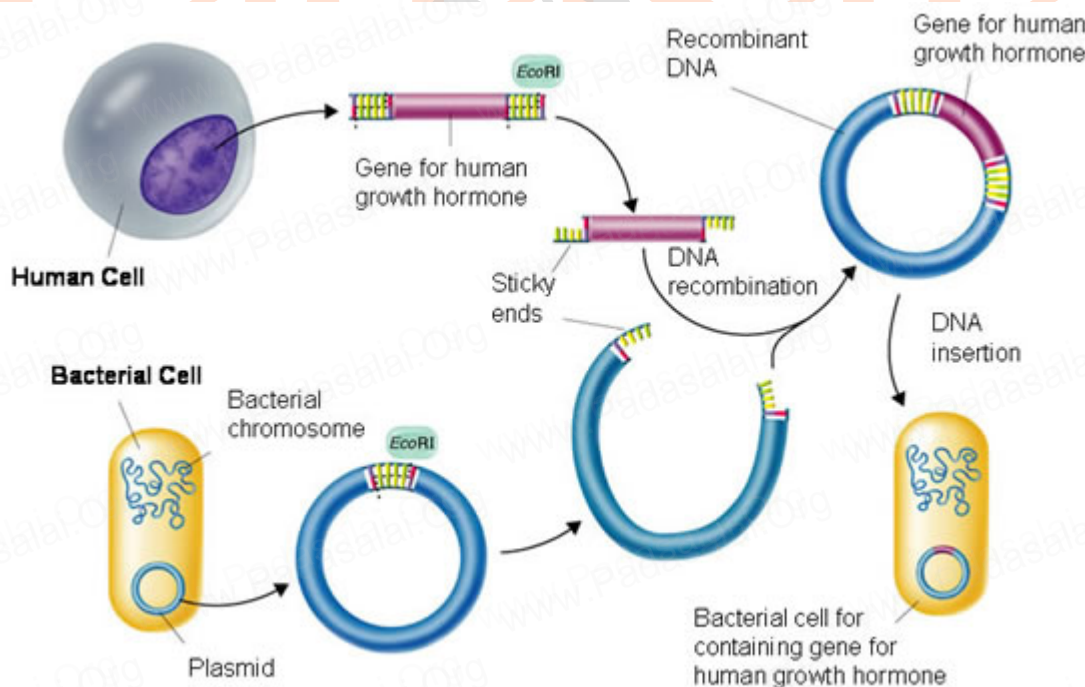


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### 9.1.2 Human Growth Hormone (hGH)

- ✎ The peptide hormones secreted by the **pituitary gland** that helps in the growth and development by increasing the uptake of amino acids and promoting protein synthesis.
- ✎ **Deficiency** of human growth hormone **causes dwarfism**, which could be treated by injecting hGH extracted from the human pituitary glands.
- ✎ Using recombinant DNA technology hGH can be produced.
- ✎ The gene for hGH is isolated from the **human pituitary gland cells**.
- ✎ The isolated gene is inserted into a plasmid vector and then is transferred into *E. coli*.
- ✎ The recombinant *E. coli* then starts producing human growth hormone.
- ✎ The recombinant *E. coli* are isolated from the culture and mass production of hGH is carried out by **fermentation technology**.
- ✎ A recombinant form of human growth hormone called **somatotropin** is used as a drug to treat growth disorders in children.





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### 9.1.3 Human Blood-Clotting Factor VIII

The genes for the formation of factor VIII is located in the X chromosome.

A genetic defect in the synthesis of factor VIII results in Haemophilia A, a sex-linked disease characterized by prolonged clotting time and internal bleeding.

Clotting factor VIII isolated from blood of normal human being was used in the treatment of Haemophilia A.

Requirement of large quantities of blood for this purpose and the risk of transmission of infectious diseases like AIDS is a disadvantage.

Recombinant DNA technology was used to produce Recombinant Factor VIII in the Chinese Hamster ovary and in the baby Hamster kidney cells.

More recently a cell line of human origin has been used for the first time to produce human blood clotting factor VIII.

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### 9.1.4 Interferons (IFNs)

1. Interferons are **antiviral agent**
2. It is very small protein molecules with the molecular weight of 20,000 to 34,000 Daltons. They are sensitive to proteolytic enzyme such as **trypsin**
3. Interferons are proteinaceous, antiviral, species specific substances produced by mammalian cells when infected with viruses.
4. 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.

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### 9.1.5 Recombinant Vaccines

- ☞ Recombinant DNA technology has been used to produce new generation vaccines.
- ☞ 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

1. **Subunit recombinant vaccines,**
2. **Attenuated recombinant vaccines and**
3. **DNA vaccines.**

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### 1. Subunit recombinant vaccines

Vaccines that use components of a pathogenic organism rather than the whole organism are called **subunit vaccines**.

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

### 2. Attenuated recombinant vaccines

This includes genetically modified pathogenic organisms (bacteria or viruses) that are made nonpathogenic and are used as vaccines.

### 3. DNA Vaccines

Genetic immunization 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.

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### 9.2 Gene Therapy

- ★ The treatment of **genetic disease** by introducing proper genes into patient's cells is called **gene therapy**
- ★ 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.

The **two** approaches to achieve gene therapy

1. **Somatic cell** and (stem cell)
2. **Germ line gene therapy**.

### 9.3 Stem Cell Therapy

- ✳ Treatment of genetic disease by introducing a remedial gene into stem cell is called **somatic cell gene therapy**.
- ✳ The remedial gene is introduced into liver cells, spleen cells, muscle cells or blood cells.

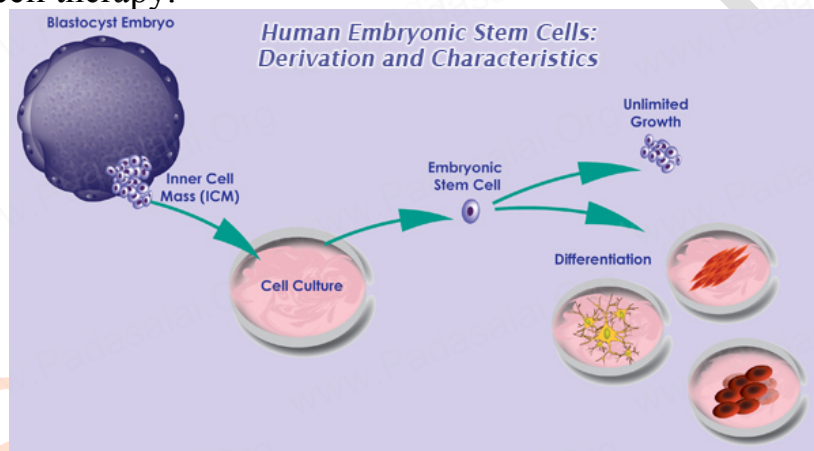
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In mammals there are two main types

They are

1. Stem cells – embryonic stem cells (ES cells) and
2. Adult stem cells.

- \* The treatment of genetic disease by introducing a proper remedial gene into cells of 2-10 days old embryo is called **embryo gene therapy** or **fetal gene therapy**.
- \* This method was devised by A. handyside et al. in 1993 to cure cystic fibrosis.
- \* If a gene is introduced into somatic cells of an adult patient, it is called patient cell therapy.



- \* 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.

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1. **Totipotency (Toti-total)** is the ability of a single cell to divide and produce all of the differentiated cells in an organism.
2. **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.
3. **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.,

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4. **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.
5. **Unipotency (Uni- Single)** refers to the ability of the stem cells to differentiate into only one cell type.

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### 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.

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.

**Table 9.1 Differentiation between somatic cell gene therapy and germ line gene therapy**

SOMATIC CELL GENE THERAPY	GERM LINE GENE THERAPY
<ul style="list-style-type: none"> <li>▪ Therapeutic genes transferred into the <b>somatic cells</b>.</li> <li>▪ Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc.</li> <li>▪ <b>Will not be inherited</b> later generations.</li> <li>▪ At present all researches directed to correct genetic defects in somatic cells.</li> </ul>	<ul style="list-style-type: none"> <li>▪ Therapeutic genes transferred into the <b>germ cells</b>.</li> <li>▪ Eg. Genes introduced into eggs and sperms.</li> <li>▪ <b>It is heritable</b> and passed on to later generations.</li> <li>▪ For safety, ethical and technical reasons, it is not being attempted at present.</li> </ul>

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### 9.4 Molecular Diagnostics

- ◆ Molecular diagnostics is a collection of techniques used to analyses biological markers in the genome and proteome. The



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individual's genetic code and how their cells express their genes as proteins by applying molecular biology to **medical testing**.

- ◆ Early detection of the disease is **not possible** using **conventional diagnostic methods** like microscopic examinations, serum analysis and urine analysis.

**Recombinant DNA technology,**

Some of the techniques that are reliable and help in early diagnosis.

They are

1. Polymerase Chain Reactions (PCR) and
2. Enzyme Linked Immunosorbent Assay (ELISA)

**1. 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.

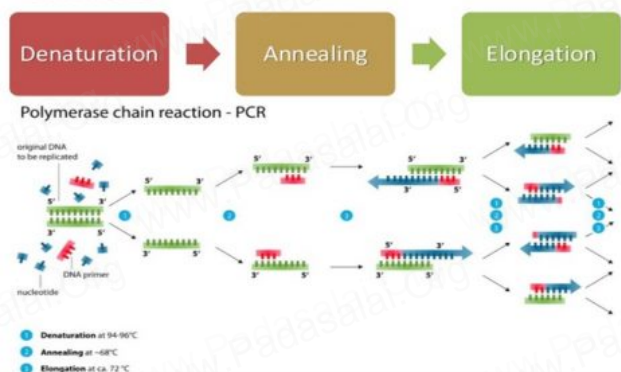
The technique was developed by **Kary Mullis** (Nobel laureate, 1993) in the year 1983.

The three steps involved in PCR

1. Denaturation,
2. Renaturation or primer annealing and
3. Synthesis or primer extension.

**Polymerase Chain Reaction (PCR)**

**STAGES**



**Applications of PCR**

1. PCR is a valuable tool for diagnosis and monitoring retroviral infections – eg. Tuberculosis by *Mycobacterium tuberculosis*.
2. Several virally induced cancers, like cervical cancer caused by **Papilloma virus** can be detected by PCR.

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3. PCR technique is also used to detect sex-linked disorders in fertilized embryos.
4. PCR is very important in the study of **evolutions**, more specifically **phylogenetic**.
5. PCR technique can also be used in the field of forensic medicine. A single molecule of DNA from blood stains, hair, and semen of an individual is adequate for amplification by PCR.
6. The **amplified DNA** is used to develop DNA fingerprint which is used as an important tool in forensic science. Thus,
7. PCR is very useful for **identification of criminals**.
8. PCR is also used in **amplification of specific DNA** segment to be used in gene therapy.
9. PCR is also employed in the prenatal diagnosis of inherited diseases by using chorionic villi samples or cells from amniocentesis.
10. Diseases like **sickle cell anemia,  $\beta$ -thalassemia and phenylketonuria** can be detected by PCR in these samples.

## **2. 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.,

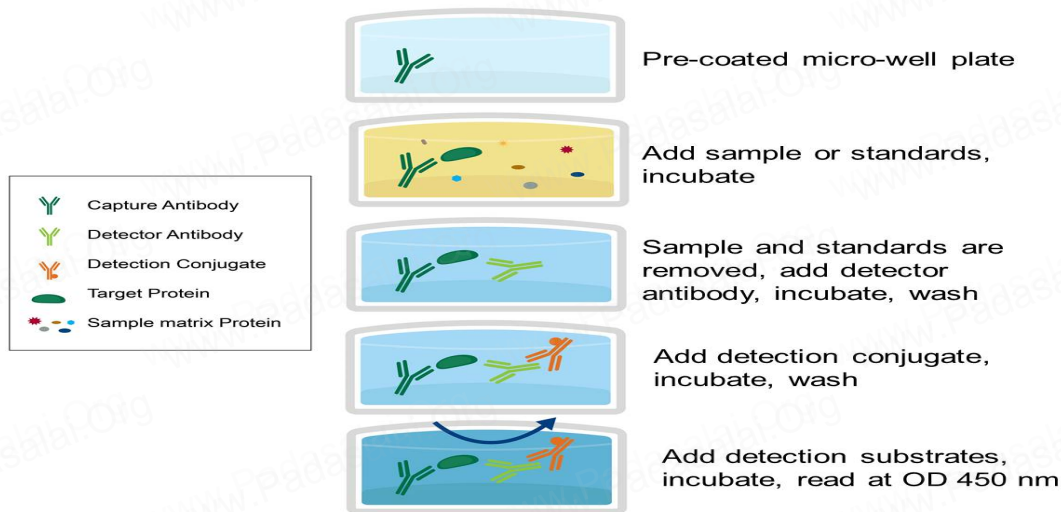
✚ It is a very important diagnostic tool to determine if a person is HIV positive or negative.

✚ 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.

There are **four** kinds of ELISA namely,

1. Direct ELISA,
2. Indirect ELISA,
3. Sandwich ELISA and
4. Competitive ELISA.

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- 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.

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## 9.5 Transgenic Animals

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.

### Transgenesis

**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 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.

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### 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.

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### 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.
- ★ There are many types of **biological products** approved for use
  - They are
    1. Therapeutic proteins,
    2. Monoclonal antibodies and
    3. Vaccines.
- ★ Hormones and antibodies are produced commercially, primarily for the medical industry.
- ★ Antibodies are substances that react against the disease causing antigens and these can be produced using **transgenic animals** as bioreactors.

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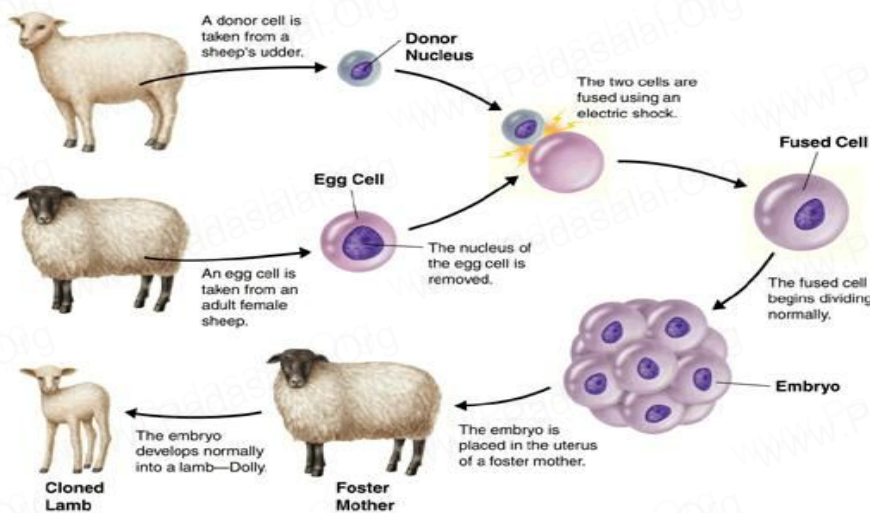
### 9.7 Animal Cloning

- ✧ 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.



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- ✳ 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.



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### Advantages and Disadvantages of Cloning Animals

Advantages	Disadvantages
High-value animals, for example cows giving high milk yield, can be cloned in large numbers	High-value animals are not necessarily produced with animal welfare in mind. Some strains of meat-producing chickens have been developed that are unable to walk
Rare animals can be cloned to preserve the species	As with plants – excessive genetic uniformity in a species makes it unlikely to be able to cope with, or adapt to, changes in the environment
Genetically modified animals – for example sheep that produce pharmaceutical chemicals in their milk – can be quickly reproduced	It is still unclear whether animals cloned using the nuclear material of adult cells will remain healthy in the long term. Dolly was put down at 6 years old due to lung cancer caused by a virus, although post-mortem showed nothing unusual

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### 9.8 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.
- ✎ The evaluation of animals is very important but a complex issue.
- ✎ 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**.

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**ALL THE BEST**

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