## Biotechnology Lectures

Biotechnology is the combination of biology+ technology =anything which involves living organisms to manufacture and produce industrial process.

Biotechnology is the use of living systems and organisms, such as bacteria or yeasts, or biological substances, such as enzymes, to develop or make useful products, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use . Depending on the tools and applications, it often overlaps with the (related) fields of bioengineering and biomedical engineering.

Biotech is commonly used in HGP (Human Genome Project) where 3 billion nucleotides are decoded. By doing this, scientists are able to discover new treatments and cures for some chronic illness such as alzhimer's disease and tuberculousis.

For thousands of years, human kind has used biotechnology in agriculture, food production, and medicine . The term itself is largely believed to have been coined in 1919 by Hungarian engineer Károly Ereky . In the late 20th and early 21st century, biotechnology has expanded to include new and diverse sciences such as genomics, recombinant gene technologies, applied immunology, and development of pharmaceutical therapies and diagnostic tests .

# History of biotechnology

Indeed, the cultivation of plants may be viewed as the earliest biotechnological enterprise. Agriculture has been theorized to become the dominant way of producing food since the Neolithic Revolution . Through early biotechnology, the earliest farmers selected and bred the best suited crops, having the highest yields, to produce enough food to support a growing population. As crops and fields became increasingly large and difficult to maintain, it was discovered that specific organisms and their by-products could effectively fertilize, restore nitrogen, and control pests. Throughout the history of agriculture, farmers have inadvertently altered the genetics of their crops through introducing them to new environments and breeding them with other plants — one of the first forms of biotechnology.

These processes also were included in early fermentation of beer. These processes were introduced in early Mesopotamia ,Egypt, China and India, and still use the same basic biological methods. In brewing, malted grains (containing enzymes) convert starch from grains into sugar and then adding specific yeasts to produce beer. In this process, carbohydrates in the grains were broken down into alcohols such as ethanol. Later other cultures produced the process of lactic acid fermentation which allowed the fermentation and preservation of other forms of food, such as soy sauce. Fermentation was also used in this time period to produce leavened bread. Although the process of fermentation was not fully understood until Louis Pasteur's work in 1857, it is still the first use of biotechnology to convert a food source into another form.

In the early twentieth century scientists gained a greater understanding of microbiology and explored ways of manufacturing specific products. In 1917, Chaim Weizmann first used a pure microbiological culture in an industrial process, that of manufacturing corn starch using Clostridium acetobutylicum, to produce acetone, which the United Kingdom desperately needed to manufacture explosives during World War I.

Biotechnology has also led to the development of antibiotics. In 1928, Alexander Fleming discovered the mold Penicillium. His work led to the purification of the antibiotic compound formed by the mold by Howard Florey, Ernst Boris Chain and Norman Heatley - to form what we today know as penicillin. In 1940, penicillin became available for medicinal use to treat bacterial infections in humans.

The field of modern biotechnology is generally thought of as having been born in 1971 when Paul Berg's (Stanford) experiments in gene splicing had early success. Herbert W. Boyer (Univ. Calif. at San Francisco) and Stanley N. Cohen (Stanford) significantly advanced the new technology in 1972 by transferring genetic material into a bacterium, such that the imported material would be reproduced. The commercial viability of a biotechnology industry was significantly expanded on June 16, 1980, when the United States Supreme Court ruled that a genetically modified microorganism could be patented in the case of Diamond v. Chakrabarty. Indian-born Ananda Chakrabarty, working for General Electric, had modified a bacterium (of the Pseudomonas genus) capable of breaking down crude oil, which he proposed to use in treating oil spills. (Chakrabarty's work did not involve gene

manipulation but rather the transfer of entire organelles between strains of the Pseudomonas bacterium.

Revenue in the industry is expected to grow by 12.9% in 2008. Another factor influencing the biotechnology sector's success is improved intellectual property rights legislation—and enforcement—worldwide, as well as strengthened demand for medical and pharmaceutical products to cope with an ageing, and ailing, U.S. population. Rising demand for biofuels is expected to be good news for the biotechnology sector, with the Department of Energy estimating ethanol usage could reduce U.S. petroleum-derived fuel consumption by up to 30% by 2030. The biotechnology sector has allowed the U.S. farming industry to rapidly increase its supply of corn and soybeans—the main inputs into biofuels—by developing genetically modified seeds which are resistant to pests and drought. By boosting farm productivity, biotechnology plays a crucial role in ensuring that biofuel production targets are met.

# Fields of Biotechnology

The wide concept of "biotech" or "biotechnology" encompasses a wide range of procedures for modifying living organisms according to human purposes, going back to domestication of animals, cultivation of plants, and "improvements" to these through breeding programs that employ artificial selection and hybridization. Modern usage also includes genetic engineering as well as cell and tissue culture technologies.

In many instances, it is also dependent on knowledge and methods from outside the sphere of biology including: bioinformatics, a new brand of computer science ,bioprocess engineering ,biorobotics, and chemical engineering

Conversely, modern biological sciences (including even concepts such as molecular ecology) are intimately entwined and heavily dependent on the methods developed through biotechnology and what is commonly thought of as the life sciences industry.

- ***Bio-pharmaceutical***

Bio-pharmaceutical companies are involved in manufacturing medicines. Products developed by pharmaceutical companies include vaccines to control the spread of

diseases, products which help in the diagnoses of diseases and, of course, products which treat diseases.

## *Forensics*

Forensic biotechnology companies are involved with forensic identification using DNA. Often, forensic bio-technologists work for law enforcement or a company which does DNA testing for law enforcement. The role of forensic scientists is to test forensic evidence and determine how it relates to human identification. Some examples of forensic identification include identifying suspects whose DNA matches the crime scene, establishing paternity or exonerating those wrongly accused.

## *Environmental Biotechnology*

Environmental biotechnology is a hugely expanding industry in the 21st century. Environmental biotechnology is studying the natural environment and finding new and better ways to produce renewable energy. According to the International Society for Environmental Biotechnology (ISEB), their mission is to find "a natural way of addressing environmental problems ranging from identification of biohazards to bioremediation techniques for industrial, agricultural and municipal effluents and residues."

## *Bioprocessing*

Bioprocessing involves using microorganisms and enzymes to develop products. Bioprocessing has been used for hundreds of years to make bread from yeast, or yogurt from cultures. Recently, biotechnologists have used bioprocessing in order to produce renewable energy fuels such as ethanol or bio-diesel. Other examples of bioprocessing include stem cell therapy, gene therapy and vaccines.

## ***Biotechnology in Space***

Biotechnology has been a powerful tool for studying how organisms respond to low gravity and other environmental conditions found in outer space. Scientists have found that microorganisms exhibit genetic changes in orbit. Biotech is also being applied to develop horticultural techniques that might someday be used on the space station or on another planet.

# Uses of Biotechnology:

Biotechnology can be used for hundreds of things. It's already made it's impact on medicine, the environment, edibles, clothing, agriculture .practicallye verything The manufacture of beer, cheese, cottage cheese, wine and bread all utilize biotechnology, High Yileding Variety (HYV) seeds have been developed due to biotechnology. Also, with newer discoveries in this field, better and more eco-friendly fertilizers, manures, pesticides, weedicides and insecticides are being developed. Biotech might show us the way to a new, renewable source of energy. Genetically modified organisms can be used to control or cure certain diseases. Also,the use of biological substances or techniques to engineer or manufacture a prod uct or substance, as when cells thatproduce antibodies are cloned in order to study thei r effects on cancer cells

The use of a living organism to solve an engineering problem or perform an industrial task. Using bacteria that feed onhydrocarbons to clean up an oil spill is one example o f biotechnology .

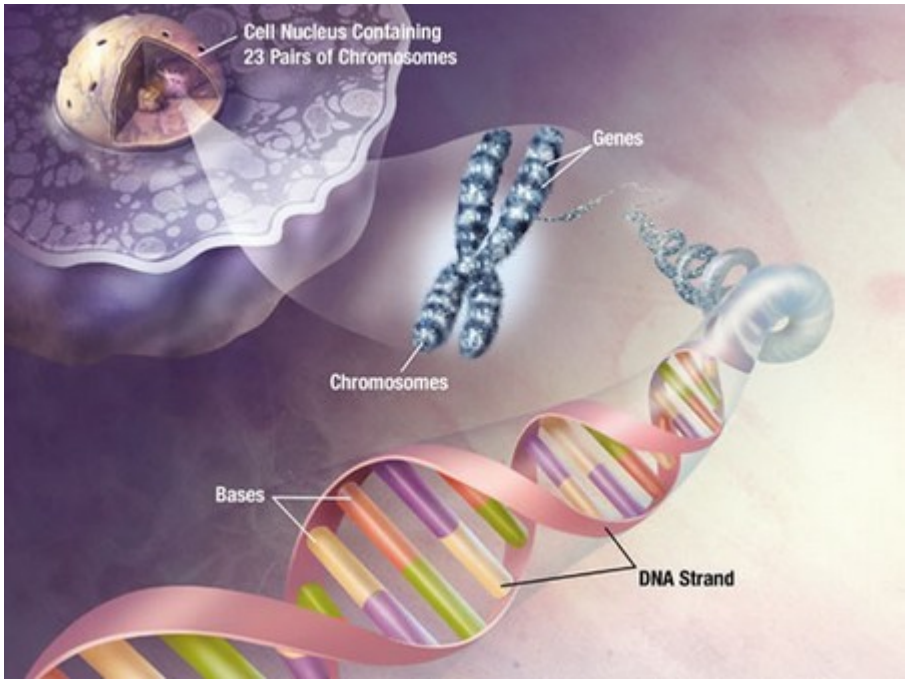
**Artificial Insemination**

Artificial insemination (AI) is an insemination process wherein sperm is unnaturally positioned into a woman's cervix or uterus. During artificial insemination, ovarian kits, ultrasounds and blood tests are used to monitor the menstrual cycles of the woman. For the implantation of semen (liquid) it is washed in the laboratories as a result of which the chances of fertilization are increased during the process of removal of unimportant, potentially damage causing chemicals. The semen is inserted into the woman, and if the procedure is successful, she becomes pregnant.

**Genetic Engineering**

Genetic engineering is the use of different methods to operate the deoxyribonucleic acid (DNA) of cells to produce the biological products or to change the hereditary traits. Techniques used include using needles to insert DNA into an ovum, hybridomas (hybrid ofcancer cells and of the cells that make the desired antibody) and the recombinant DNA, wherein the DNA of a desired gene is inserted into the DNA of bacterium, after that the bacterium multiplies itself, producing more of the desired gene. Other type is polymerase chain reaction (PCR) in which DNA quickly replicate

to create a huge analyzable sample. The process makes perfect copies of DNA fragments and is used in DNA fingerprinting.




**Cloning**

Cloning is a term used to refer to the creation of offspring by means of asexual reproduction. With the help of cloning, we can make identical copies of animals such as sheep, Dolly. When this technique was introduced scientist made the dolly sheep with the help of cloning in which they replace the nucleus with the other one and in this way we get the identical copies of animal. But it considered as an immoral activity which yet considered unethical for the cloning of human beings. However, there are several plants which have been produced through cloning over centuries.

## Branches of Biotechnology

There are several branches of Biotechnology. They are classified as:

**A) Blue Biotechnology**:

This branch of biotechnology helps to control the marine organisms and water borne organisms. It is a process which has to do with marine or underwater environment. The use of this biotechnology is very rare. Blue biotechnology is used to protect the marine organisms from harmful diseases underwater.

**B) Bioinformatics**:

Bioinformatics is the combination of computer and biotechnology. It helps in finding the analysis of datas related to Biotechnology. It is used for various purposes like drugs, for the development of medicines; it is also used to improve the fertility of crops and plants and also for pest, drought and it is resistance to diseases. Bioinformatics is known and referred by the term â€œcomputational biologyâ€It plays an important and a vital role in areas like Functional genomics, structural genomics and proteomics these areas contribute a lot and become a key contributor to Biotechnology and pharma sector.

**C) Green Biotechnology**:

Green Biotechnology is the term used for the agricultural sector. With the help of the process called the Micropropagation (a practice of producing larger number of plants through the existing stock of plants) which helps in selecting the right quality of plants and crops. Also with the help of Transgenic plants (plants whose DNA is modified); this design of transgenic plants helps to grow in a specified environment with the help of certain chemicals.

**D) Red Biotechnology**:

Red biotechnology is referred to as Medical Biotechnology. It is used for the production of drugs and antibiotic medicines. It also helps to create or design organisms. Through the process of genetic manipulation it helps to cure genetic issues in organisms. It also helps in analysing diseases in organisms. It also helps in developing new ways of diagnosis by performing tests. With the help of stem cell therapy it helps the organs to grow and it also cures the damaged issues in organisms.

**E) White Biotechnology**:

White Biotechnology is also called and known by the name Industry Biotechnology. This kind of biotechnology is used and applied in industries and its processes. The various uses of this Biotechnology includes; biopolymers (Plastics) Substitutes, new invention of vehicle parts and fuels for the vehicles, invention of fibres for the clothing industry, it is also involved in developing new chemicals and the production process

# Medical biotechnology

Medical biotechnology is the fusion of genetics, cell biology and many other sciences in order to further advances in medicine .

Why is medical biotechnology important?

- The main reason for medical biotechnology is to prolong life.
- Other reasons are to ease suffering of palliative care patients or to increase accessibility for people with disabilities.

## insulin production

Synthetic human insulin was the first golden molecule of the biotech industry and the direct result of recombinant DNA technology. Currently, millions of diabetics worldwide use synthetic insulin to regulate their blood sugar levels. Synthetic insulin is made in both bacteria and yeast.

The process of insulin production using Biotechnology involves the following steps:

### Isolation of the gene:

The gene responsible for producing human insulin is located on chromosome 11. The gene is fairly small and consists of two polypeptide chains, an A chain which codes for 21 amino acids and a B chain which codes for 30 amino acids. The both chains are linked by a disulphide bond .

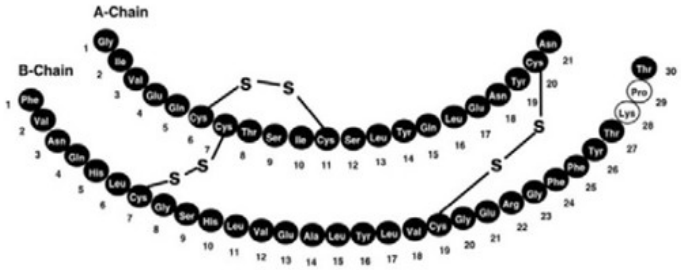


Figure 1 illustrating the chemical structure of insulin gene

The enzyme, restriction endonuclease splices the DNA at specific nucleotide bases in order to isolate the gene. This process is called restriction digestion and results in the formation of sticky or blunt ends.

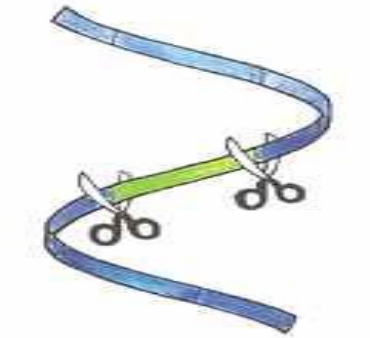


*Figure 2illustrating the splicing of the DNA with the restriction endonuclease enzyme.*

Another method may involve using the mRNA for the insulin gene and reverse transcriptase to produce a complementary/ copy DNA (cDNA) strand.

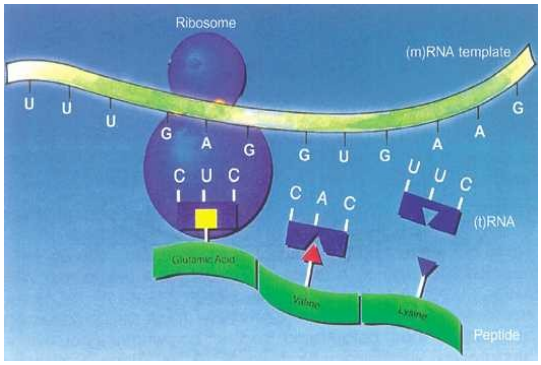



*Figure 3 showing translation of mRNA*

**Preparation of the target DNA**

A plasmid, which is a circular piece of DNA, is removed from the bacteria *Escherichia coli* and purified. The same restriction endonuclease enzyme is used to splice the plasmid ring .This produces complementary sticky ends to which the insulin gene can be attached . The plasmid is referred to as a vector.

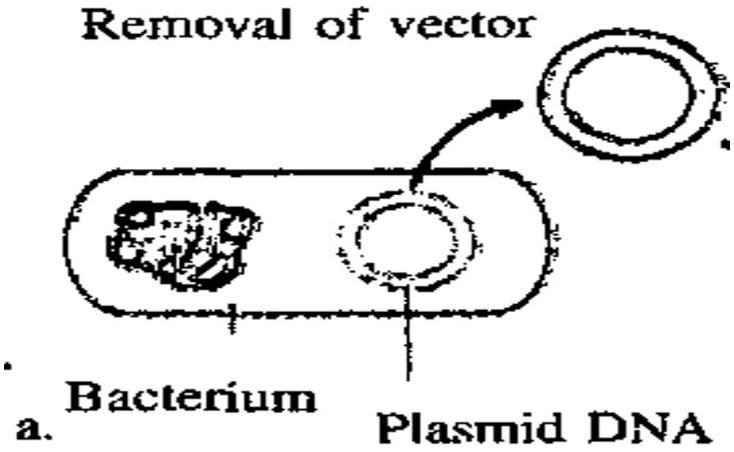



*Figure 4 illustrating the removal of the plasmid from the bacterium*

## Insertion of DNA into the plasmid

The human insulin gene is ligated next to the *lacZ* gene on the plasmid, thus forming a recombinant plasmid. The A and B chains are inserted separately into different plasmids. LacZ encodes for B-galactosidase and is widely used in recombinant DNA procedures. This is due to the fact that it is easy to locate and splice, allowing the insulin produced to be readily removed so that it does not get lost in the bacterium's DNA. Adjacent to this gene, is the amino acid methionine, which .initiates insulin formation

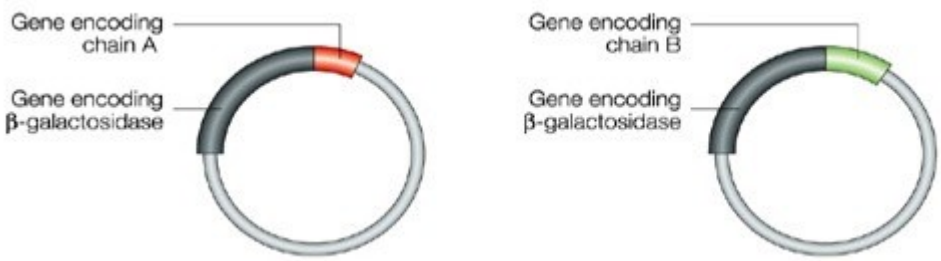



*Figure 5 illustrating the recombinant plasmids*

## Insertion of plasmids back into bacterial cell

The bacterial cell is made competent either by electroporation or by using a cold liquid, such as CaCl2 (calcium chloride) to temporarily induce a more permeable cell membrane. The recombinant plasmids and the bacterial cells are then mixed together. Plasmids enter the bacteria in a process called transformation.

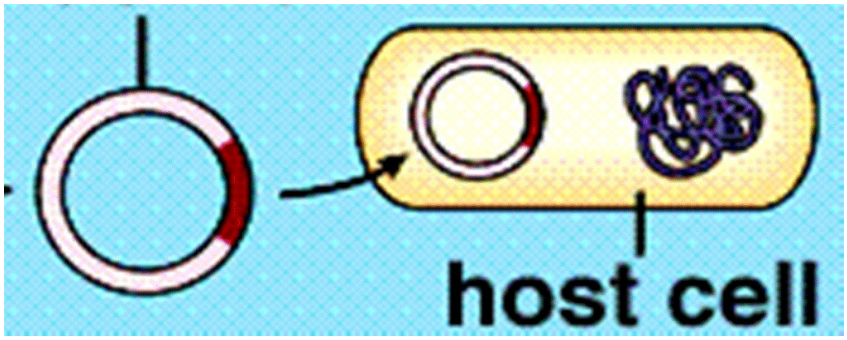



*Figure 6 illustrating the plasmid being inserted back into the bacterial host cell.*

## Cloning of the plasmid

The recombinant plasmid replicates autonomously within the host cell resulting in the creation of numerous identical copies.

## Reproduction of bacterial host cells

At optimal temperatures within large tanks, in manufacturing industries, the bacterial cells reproduce mitotically every 20 minutes. Each daughter cell contains a recombinant plasmid and expresses the gene for human insulin .

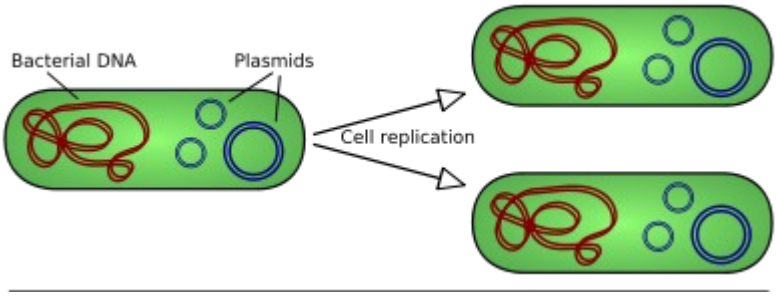



*Figure 7 showing replication of bacterial cell.*

## Production and end treatment of the protein

After cloning, the bacterial cells are removed from the tanks and lysed in order to extract the protein. A popular method involves first adding a mixture of lysozome that digests the outer layer of the cell wall, then adding a detergent mixture that separates the fatty cell wall membrane. The bacterium's DNA is then treated with cyanogen bromide, a reagent that splits protein chains at the methionine residues. This separates the insulin chains from the rest of the DNA.

The two chains are then mixed together and joined by disulfide bonds through the reduction-reoxidation reaction. An oxidizing agent is added and then the batch is placed in a centrifuge.

The mixture is then purified so that only the insulin chains remain. This can be done using several chromatography or separation techniques. The insulin batches are usually tested to ensure that none of the bacteria's *E. coli* proteins are mixed in with them. A marker protein that can detect *E. coli* DNA is used .

**Human Growth Hormone**

Growth hormone (GH or HGH), also known as somatotropin or somatropin, is a peptide hormone that stimulatesgrowth, cell reproduction and regeneration in humans and other animals. It is a type of mitogen which is specific only to certain kinds of cells. Growth hormone is a 191-amino acid, single-chain polypeptide that is synthesized, stored, and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland.

Prior to the use of recombinant DNA technology to modify bacteria to produce human growth hormone, the hormone was manufactured by extraction from the pituitary glands of cadavers, as animal growth hormones have no therapeutic value in humans. Production of a single year's supply of human growth hormone required up to fifty pituitary glands, creating significant shortages of the hormone. In 1979, scientists at Genentech produced human growth hormone by inserting DNA coding for human growth hormone into a plasmid that was implanted in Escherichia coli bacteria. The gene that was inserted into the plasmid was created by reverse transcription of the mRNA found in pituitary glands to complementary DNA. HaeIII, a type of restriction enzyme which acts at restriction sites "in the 3' noncoding region" and at the 23rd codon in complementary DNA for human growth hormone, was used to produce "a DNA fragment of 551 base pairs which includes coding sequences for amino acids 24–191 of HGH." Then "a chemically synthesized DNA 'adaptor' fragment containing an ATG initiation codon..." was produced with the codons for the first through 23rd amino acids in human growth hormone. The "two DNA fragments... [were] combined to form a synthetic-natural 'hybrid' gene." The use of entirely synthetic methods of DNA production to produce a gene that would be translated to human growth hormone in Escherichia coli would have been exceedingly laborious due to the significant length of the amino acid sequence in human growth hormone. However, if the cDNA reverse transcribed from the mRNA for human growth hormone were inserted directly into the plasmid inserted into the Escherichia coli, the bacteria would translate regions of the gene that are not translated in humans,

thereby producing a "pre-hormone containing an extra 26 amino acids"which might be difficult to remove.

## Production of Protropin

1. A gene that produces growth hormone in humans is isolated.
2. The growth hormone production gene is inserted into the DNA of E.Coli bacteria.
3. The bacteria recognise the inserted DNA as its own DNA and begin to produce human growth hormone.
4. The bacteria multiply and produce the growth hormone in a culturing media.
5. The growth hormone is extracted and purfied and is then ready to be injected into children with GHI.

## Stem cell

- A stem cell is a cell that has the potential to become any cell type in the human body. Everyone has stem cells, but they are very hard to access.
- The easiest place to get stem cells is from an embryo. Stem cells are introduced into a damaged area of the body where, under the right conditions, will replace the damaged area. Often times stem cells are grown in a lab first to ensure the right conditions and then placed into a sick person Stem cells are currently being tested to treat everything from Crohn's disease to baldness! The main areas where stem cells have proven their worth is in bone marrow transplants, replacing damaged heart tissue after a heart attack and replacing damaged nerve tissue which gives hope to anyone who has had a spinal cord injury .

**Monoclonal antibodies**

They are so called because they are clones of an individual parent cell., antibodies are specific proteins that target pathogens invading the body . This technology is used primarily to fight off cancer cells as these monoclonal antibodies can be “trained” to target markers that show up on cancer cells. The mAbs will then destroy the cancer cell and go looking for more .

- Steps in making them:

1. Human antibody genes are put into a mouse.
2. Mouse is infected causing it to make human antibody producing cells (B-cells).
3. These cells are removed from the mouse and fused with a tumor cell.
4. a tumor cell that is constantly producing antibodies and more cells like itself.

## Perspective 17.1 Monoclonal Antibodies

In 1975, an exciting breakthrough occurred in immunology. Georges Köhler and Cesar Milstein developed techniques that fused normal antibody-producing B lymphocytes with malignant plasma cells (myeloma tumor cells), resulting in clones of cells they termed **hybridomas**. Since these hybridomas are clones, they produce antibodies with a single specificity that, therefore, are known as **monoclonal antibodies (figure 1)**.

Plasma cells produce large amounts of antibody, and when they become malignant myeloma cells grow profusely and indefinitely. Special myeloma cells are used to make hybridomas; they have lost the ability to make their own specificity of antibody but have retained the ability to produce large amounts of immunoglobulin. The normal B cell in the hybridoma supplies the genes for the specific antibody to be produced; the myeloma cell supplies the cellular machinery, the rough endoplasmic reticulum, for producing the antibodies.

Usually, when an animal is injected with an immunizing agent, it responds by making a variety of antibodies directed against different epitopes on the antigen. Therefore, even though there is a single antigen, the result is a mixture of different antibodies. When these antisera are used in immunological tests, standardizing the results is difficult, since there are differences each time the antiserum is made. Monoclonal antibodies, however, will be of the same immunoglobulin class and have the same variable regions and, thus, the same specificity and other characteristics. With such specificity, tests can be standardized much more easily and with greater reliability.

It was hoped that monoclonal antibodies would also become a useful therapeutic tool. In theory, it should be possible to make monoclonal antibodies that are specific for, say, a cancer cell. Radioactive materials that destroy cancer cells could be attached to the monoclonals making a sort of "magic bullet." These antibodies would search and find the cancer cells and attach specifically to them, allowing the radioactive material to destroy the cells. In reality, many problems have arisen when the monoclonal antibodies have been used to treat humans. One problem was that mouse cells were used to produce the hybridomas, and humans reacted to the mouse antigens on the hybridoma antibodies, causing them to be rapidly removed from the body. This limited the effectiveness of the monoclonal antibodies to one or a few doses. This and many other unforeseen difficulties are being addressed now, with some encouraging results. For example, genetic engineering is being used to construct antibody genes from human DNA that can be put into hybridomas to produce monoclonal antibodies useful in treating humans.

In the laboratory, monoclonal antibodies are the basis of a number of diagnostic tests. For example, monoclonal antibodies against a hormone can detect pregnancy only 10 days after conception. Specific monoclonal antibodies are used for rapid diagnosis of hepatitis, influenza, herpes simplex, and chlamydia infections. Köhler and Milstein won the Nobel Prize in 1984 for their work.

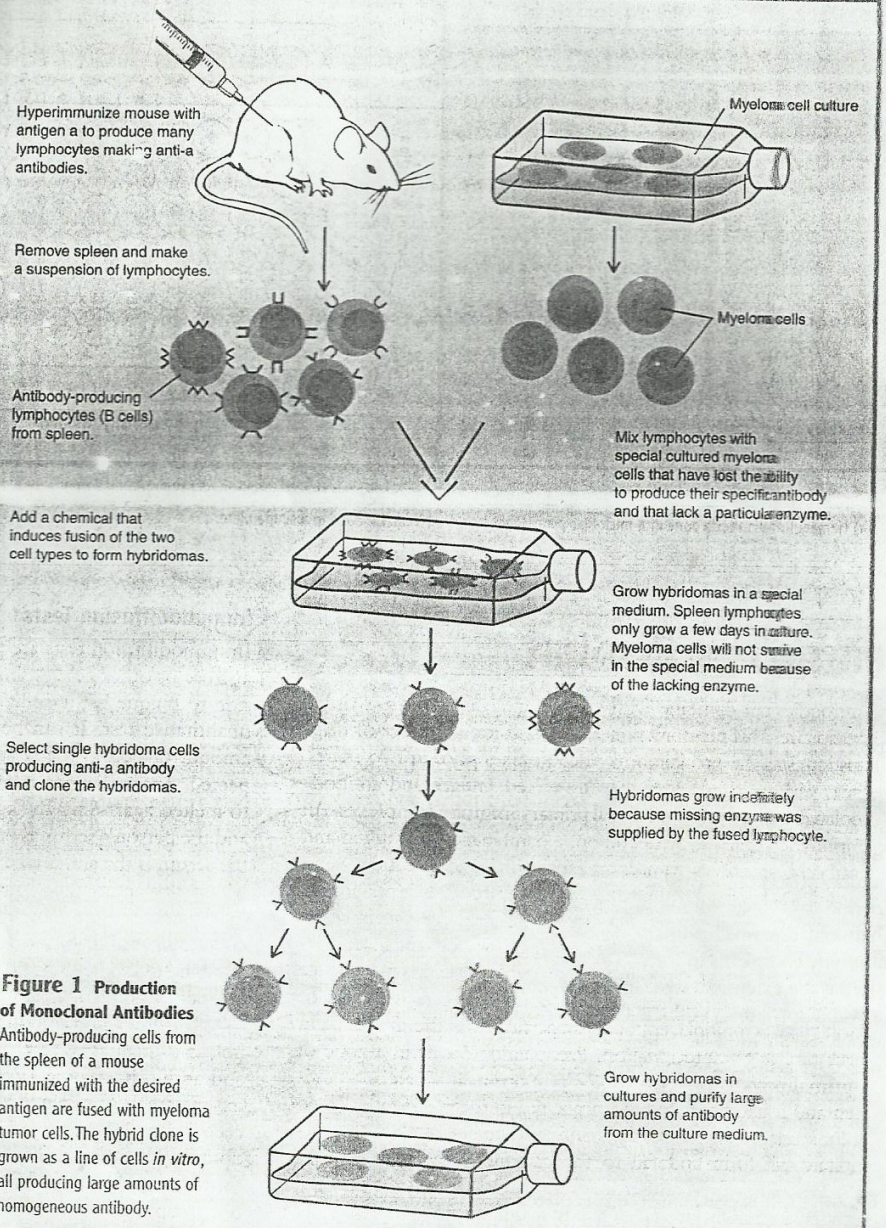



**Figure 1 Production of Monoclonal Antibodies** Antibody-producing cells from the spleen of a mouse immunized with the desired antigen are fused with myeloma tumor cells. The hybrid clone is grown as a line of cells *in vitro*, all producing large amounts of homogeneous antibody.

## Vaccine

Vaccination involved deliberate exposure to antigen under conditions where disease should not result .It provides one of the beast means for preventing ,rather than treating infectious disease. Vaccination has succeeded in eliminating the smallpox virus worldwide .

Vaccine the main or sole active ingredient consists of killed microorganisms, non virulent microorganisms, microbial products (e.g., toxins), or microbial components that have been purified. All these active ingredients are antigens: substances that can stimulate the protective immunity ,which may involve secretion neutralizing specific antibodies or production memory CTL or TH1 cells . Such stimulation leaves the immune system prepared to destroy bacteria and viruses whose antigens correspond to the antibodies it has learned to produce. Although conventionally produced vaccines are generally harmless, some of them may, rarely, contain infectious contaminants. Vaccines whose active ingredients are recombinant antigens do not carry this slight risk. More than 350 million persons worldwide are infected with the virus that causes hepatitis B, a major cause of chronic inflammation of the liver, cirrhosis of the liver, and liver cancer. Hepatitis B kills a million people each year worldwide .The first hepatitis B vaccine available in the U.S. was made with derivatives of plasma from persons with chronic HBV infections. A recombinant vaccine—whose sole active ingredient is a recombinant (and thus uncontaminated) antigen—has replaced it. Use of this vaccine is very cost-effective—especially in North America, since interferon treatment of hepatitis B is very expensive.

**Recombinant HB Vaccine Production**

Hepatitis B (HB) is one of the most common infectious diseases known to man.

The World Health Organization estimates that there are as many as 285 million chronic carriers of this virus worldwide. Traditional vaccines use a weakened or killed form of a virus to force the body to develop antibodies that are strong enough to combat the virus.

**Recombivax HB** was approved as a hepatitis B prevention vaccine in July 1986.

Using recombinant DNA technology, Recombivax HB uses the surface antigen of the virus that stimulates the production of protective antibodies which combat the HB virus.

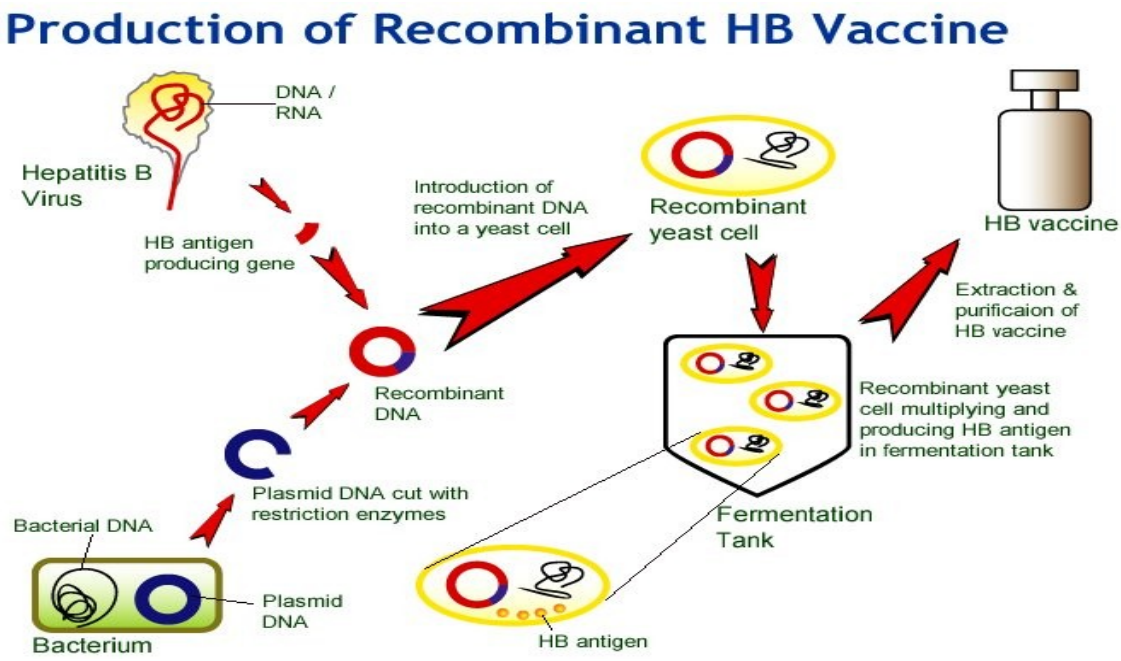
**Production of Recombivax HB**

1. HB antigen producing gene is isolated from the HB virus.
2. A plasmid DNA is extracted from a bacterium and is cut with restriction enzyme forming the plasmid vector.
3. The isolated HB antigen producing gene is inserted into the bacterial plasmid vector on forming the recombinant DNA.
4. This recombinant DNA, containing the target gene, is introduced into a yeast cell forming the recombinant yeast cell.
5. The recombinant yeast cell multiplies in the fermentation tank and produces the HB antigens.
6. The HB antigens are extracted , purified and bottled. It is ready for vaccination in humans.

**Advantage of Recombivax HB**

Recombivax HB has a significant advantage over the conventional HB Vaccine.

Since only small amounts of protein of the virus are present in the vaccine and the virus itself is not used; it is impossible for a person to become infected by the vaccine.




## Genetic testing

It is a type of medical test that identifies changes in chromosomes, genes, or proteins. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. More than 1,000 genetic tests are currently in use, and more are being developed.

Genetic tests also determine whether ornot couples are at a higher risk than the gener al population for having a child affected with a genetic disorder. Several methods can be used for genetic testing :

**Molecular genetic tests** (or gene tests) study single genes or short lengths of DNA to identify variations or mutations that lead to a genetic disorder.

**Chromosomal genetic tests** analyze whole chromosomes or long lengths of DNA to see if there are large genetic changes, such as an extra copy of a chromosome, that cause a genetic condition.

**Biochemical genetic tests** study the amount or activity level of proteins; abnormalities in either can indicate changes to the DNA that result in a genetic disorder . Genetic testing is voluntary. Because testing has benefits as well as limitations and risks, the decision about whether to be tested is a personal and complex one. A geneticist or genetic counselor can help by providing information

about the pros and cons of the test and discussing the social and emotional aspects of testing .

***: The types of genetic tests***

Genetic testing can provide information about a person's genes and chromosomes. Available types of testing include :

**1-Newborn screening :** It is used just after birth to identify genetic disorders that can be treated early in life. Millions of babies are tested each year in the United States. All states currently test infants for phenylketonuria (a genetic disorder that causes mental retardation if left untreated) and congenital hypothyroidism (a disorder of the thyroid gland ) also test for other genetic disorders.

**2-Diagnostic testing :** It is used to identify or rule out a specific genetic or chromosomal condition . In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical signs and symptoms. Diagnostic testing can be performed before birth or at any time during a person's life, but is not available for all genes or all genetic conditions . The results of a diagnostic test can influence a person's choices about health care and the management of the disorder.

**3-Carrier testing :** It is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in certain ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition.

**3-Prenatal testing :** Prenatal testing is used to detect changes in a fetus's genes or chromosomes before birth. This type of testing is offered during pregnancy if there is an increased risk that the baby will have a genetic or chromosomal disorder. In some cases, prenatal testing can lessen a couple's uncertainty or help them make decisions about a pregnancy. It cannot identify all possible inherited disorders and birth defects, however.

**4-Preimplantation testing :** Preimplantation testing, also called preimplantation genetic diagnosis (PGD), is a specialized technique that can reduce the risk of having

a child with a particular genetic or chromosomal disorder. It is used to detect genetic changes in embryos that were created using assisted reproductive techniques such as in-vitro fertilization. In-vitro fertilization involves removing egg cells from a woman's ovaries and fertilizing them with sperm cells outside the body. To perform preimplantation testing, a small number of cells are taken from these embryos and tested for certain genetic changes. Only embryos without these changes are implanted in the uterus to initiate a pregnancy.

**5-Predictive and presymptomatic testing :** Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder, but who have no features of the disorder themselves at the time of testing. Predictive testing can identify mutations that increase a person's risk of developing disorders with a genetic basis, such as certain types of cancer. Presymptomatic testing can determine whether a person will develop a genetic disorder, such as hemochromatosis (an iron overload disorder), before any signs or symptoms appear. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder and help with making decisions about medical care.

**6-Forensic testing** : It uses DNA sequences to identify an individual for legal purposes. Unlike the tests described above, forensic testing is not used to detect gene mutations associated with disease. This type of testing can identify crime or catastrophe victims, rule out or implicate a crime suspect, or establish biological relationships between people (for example, paternity).

**How is genetic testing done**

Genetic tests are performed on a sample of blood, hair, skin, amniotic fluid (the fluid that surrounds a fetus during pregnancy), or other tissue. For example, a procedure called a buccal smear uses a small brush or cotton swab to collect a sample of cells from the inside surface of the cheek. The sample is sent to a laboratory where technicians look for specific changes in chromosomes, DNA, or proteins, depending on the suspected disorder. The laboratory reports the test results in writing to a person's doctor or genetic counselor, or directly to the patient if requested.

Newborn screening tests are done on a small blood sample, which is taken by pricking the baby's heel. Unlike other types of genetic testing, a parent will usually only receive the result if it is positive. If the test result is positive, additional testing is needed to determine whether the baby has a genetic disorder.

**The Pharmacogenomics** is the study of how variations in genes affect response to medications, thereby using a patient's genetic profile to predict a drug's efficacy, guide dosage and improve patient safety. It is coined word derived from the word pharmacology and genomics. It is hence the study of the relation relationship between Pharmaceutical and genetics.

**Pharmacogenetics** is the study of inherited differences in drug metabolism and response .

The purpose of pharmacogenomic testing is to find out if a medication is right for you. A small blood or saliva sample can help determine:

- Whether a medication may be an effective treatment for you
- What the best dose of a medication is for you
- Whether you could have serious side effects from a medication

The laboratory looks for changes or variants in one or more genes that can affect your response to certain medications.

Genes are the portions of chromosomes that determine many of the traits in every living thing. In humans, genes influencerace, hair and eye color, gender, height, weight, aspects of behavior, and even the likelihood of developing certain diseases. Although some traits are a combination of genetics and environment, researchers are still discovering new ways in which people are affected by their genes .

Pharmacogenetics is the study of how people respond to drug therapy. Although this science is still new, there have been

many useful discoveries. It has long been known that genes influence the risk of developing certain diseases, or that genes

could determine traits such as hair and eye color. Genes can also alter the risk of developing different diseases. It has long been known that people of African descent were more likely to have sickle cell anemia than people of other races. People of Armenian, Arab, and Turkish heritage are more prone to familiar Mediterranean fever than people of other nationalities. Morerecently, discoveries have shown that genes

can determine other aspects of each individual, down to the level of the enzymes produced in the liver. Since these enzymes determine how quickly a drug is removed from the body, they can make major differences in the way people respond to drugs. Some of the most basic work concerns the way race and gender influence drug reactions and race and gender are genetically determined.

Women often respond differently than men to drugs at the same dose levels. For example, women are more likely to have agood response to the antidepressant drugs that act as serotonin specific reuptake inhibitors (SSRIs ) than they are to the older group of tricyclic antidepressants (the group that includes Elavil and Tofranil). Women have a greater response to some narcotic pain reliving drugs than do men, but get less relief from some non-narcoticpain medications. Women may show a greater r esponse to some steroid hormones than men do, but have a lower level ofresponse to s ome anti-anxiety medications than men.

Race may also affect the way people respond to some medications. In this case, race implies specific genetic factors that are generally, but not always, found among members of specific ethnic groups. For example, the angiotensin II inhibitor enalopril (Vasotec), which is used to lower blood pressure, works better in Caucasians than in Blacks. Carvedilol (Coreg), abeta-adrenergic blocking agent that is also used t o lower blood pressure, is more effective than other drugs in the same class when used to treat Black patients.

Genes alter responses to drugs because the genes influence many parts of the body itself. One of the simplest examples isthe gene that influences body weight. Since many drugs are soluble in body fat, people with large amounts of fat will have these drug deposited into their fat stores. This means that there are lower levels of the drug that can reach the actual organs on which they work.

In the case of gender responses to antidepressants, women show greater response to serotonin specific antidepressantsbecause women naturally have lower levels of serotonin than men do. This makes women more likely to develop a type of depression marked by low serotonin levels, but it also means that women will respond better to replacement of serotonin.

Because people of the same race carry similar genes, studies based on race were the

earliest types of pharmacogeneticstudies. One study evaluated the levels of alcohol dehydrogenase in people of different nationalities. This is an enzyme involved in the metabolism of alcohol. When people with high levels of this enzyme, or people in whom the enzyme acts morerapidly than in other people, drink alcohol, they are subject to facial flushing and slowing of the heartbeat. The activity of this enzyme is determined by genetics, and different levels can be seen in different races because these people belong to thesame gene pools. Among Asiatic people, 85% have high levels of this enzyme, compared to 20% of Swiss people, and only5-10% of Briti sh people.

Another trait that is influenced by genes is a liver enzyme, CYP2D6. This enzyme metabolizes some drugs, convert them toa form that can be removed from the body. Genes determine the level of this enzyme in the liver. People with low levels of CYP2D6 will metabolize drugs slowly. Slow metabolism means the drugs will act for a longer period of time. Slow metabolizers reaspond to smaller doses of medications that are eliminated by this enzyme, while fast metabolizers, peoplewho have a lot of the enzyme, will need larger drug doses to get the same effects. At the same time, low levels of CYP2D6means tha t people taking the drugs that are metabolized by this enzyme will have higher drug le vels, and are more likely tohave unwanted side effects.

Another enzyme that can be important in drug dosing is called 2C9, and this enzyme i s responsible for metabolizing theanticoagulant drug warfarin (Coumadin). Most peop le take warfarin in a dose of about 5 milligrams a day, but people whohave low levels of 2C9 normally require a dose of only 1-5 milligrams a week.

Yet another mechanism of drug activity is the presence or absence of a specific drug receptor site. Drugs act by binding tospecific chemicals, receptor sites, within body cells. Genes may help determine how many of these cells there are.

The action of the widely used antipsychotic drug haloperidol (Haldol) depends on its ability to bind to the dopamine (D2) receptorsite. The number of these sites are determined by genetics . In one study, 63% of patients whose genes caused a large number of these receptor sites had a response to treatment with haloperidol, while only about 29% of patients with a smallernumber of dopamine (D2) receptor sites did well on the drug.

Other genetic studies indicate that genes may affect how people respond to foods as well as to drugs. An Australian study ofosteoporosis (softening of the bones that often

occurs in elderly people), reported that separate genes may affect responseto vitamin D, calcium, and estrogen .

# Bioreactor

An apparatus, such as a fermentation chamber, for growing organisms such as bacteria or yeast that are used in the biotechnological production of substances such as pharmaceuticals, antibodies, interferon or vaccines, or for the bioconversion of organic waste. Or break down harmful ones (as in sewage)

**Fermentation** is the conversion of a carbohydrate such as sugar into an acid or an alcohol. It can refer to the use of yeast to change sugar into alcohol or the use of bacteria to create lactic acid in certain foods. This process occurs naturally in many different foods given the right conditions, and humans have intentionally made use of it for many thousands of years.

The earliest uses of fermentation were most likely to create alcoholic beverages such as mead, wine, and beer. These beverages may have been created as far back as 7,000 BCE in parts of the Middle East. The fermentation of foods such as milk and various vegetables probably happened sometime a few thousand years later, in both the Middle East and China. While the general principle is the same across all of these drinks and foods, the precise methods of achieving it, and the end results, differ.

**Beer** is made by taking a grain, such as barley, wheat, or rye, germinating and drying it, and pulping it into a mash. This mash is then mixed with hot water, and some fermentation begins. After being further treated, the liquid is transferred to a vessel, where yeast is added to the mixture. This yeast "eats" the sugar present in the mash and converts it into carbon dioxide and alcohol. After a few weeks of fermentation and a further period of conditioning, the beer is ready to be filtered and consumed.

**Wine** is created using a similar method that also involves fermentation. Grapes are crushed to release the sugar-rich juices, which are then either transferred quickly away from the skins or left to rest for a time to absorb some of the flavor, tannins, and color of the skins. Yeast is then added, and the grape juice is allowed to ferment for a number of weeks, at which point it is moved to different containers and processed at a slower rate, and eventually aged or bottled.

**Pickling foods,** such as cucumbers, may be accomplished by submerging the vegetable one wants to pickle in a salty water solution with vinegar added. Over time, bacteria create the lactic acid that gives the food its distinctive flavor and helps to preserve it. Other foods can be pickled simply by packing them in dry salt and allowing a natural fermentation process to occur.

**Milk** can also be cultured, and people have been using this process with dairy products for nearly 5,000 years. It is speculated that early dairy products, such as yogurt, was the result of a natural processes that occurred when the milk was cultured by bacteria that dwelt in skin sacks used to store dairy. Yogurt these days is made by adding a number of special bacteria, such as *L. acidophilus* and *L. bulgaricus* to milk and keeping it at the proper temperature. The bacteria begin converting the sugar in the dairy to lactic acid, eventually creating what we know as yogurt.

## Protoplast fusion

Protoplasts are the cells of which cell walls are removed and cytoplasmic membrane is the outermost layer in such cells. Protoplast can be obtained by specific lytic enzymes to remove cell wall. Protoplast fusion is a physical phenomenon, during fusion two or more protoplasts come in contact and adhere with one another either spontaneously or in presence of fusion inducing agents.

Two genetically different protoplast isolated from the somatic cells and are experimentally fused to obtain parasexual hybrid protoplasts. The hybrid protoplast contained heteroplasoic cytoplasm and two fused parent nuclei .Fusion of protoplast is relatively a new versatile technique to induce or promote genetic recombination in a variety of prokaryotic and eukaryotic cells Protoplast fusion may be used to produce interspecific or even intergeneric hybrids.Protoplast fusion becomes an important tool of gene manipulation because it breakdown the barriers to genetic exchange imposed by conventional mating systems. Protoplast fusion technique has a great potential for genetic analysis and for strain improvement . It is particularly useful for industrially useful microorganisms .

**Enzymes used for breaking of cell walls** :

For protoplast fusion it is important that the cell wall of plant and microorganisms is

degraded .So various enzymes used for this process. cellulase and pectinase or macerozyme acting on plant cell wall .Bacterial cell wall are degraded by the action of lysozyme. Fungal wall degraded by Novozyme -234 which includes glucanase and chitinase. Streptomyces cell wall degraded by action of lysozyme and achromopeptidase

**Methods of protoplast fusion**:

Protoplast fusion can be broadly classified into two categories:

**Spontaneous fusion**: Protoplast during isolation often fuse spontaneously and this phenomenon is called spontaneous fiusion .During the enzyme treatment ,protoplast from adjoining cells fuse through their plasmodesmata to form multinucleate protoplasts.

**Induced fusion**:Fusion of freely isolated protoplasts from different sources with the help of fusion inducing chemicals agents is known as induced fusion. Normally isolated protoplast do not fuse with each other because the surface of isolated protoplast carries negative charges (-10mV to -30mV ) around the outside of the plasma membrane. And thus their is a strong tendency in the protoplast to repel each other due to their same charges .So this type of fusion needs a fusion inducing chemicals which actually reduce the electronegativity of the isolated protoplast and allow them to fuse with each others.

The isolated protoplast can be induced to fuse by three ways;

**Mechanical fusion**:In this process the isolated protoplast are brought into intimate physical contact mechanically under microscope using micromanipulator or perfusion micropipette.

**Chemofusion**: Several chemicals has been used to induce protoplast fusionsuch as sodium nitrate ,polyethylene glycol,Calcium ions(Ca++ ). Chemical fusogens cause the isolated protoplast to adhere each other and leads to tight agglutination followed by fusion of protoplast.

**Electrofusion**: Recently ,mild electric stimulation is being used to fuse protoplast .In this two glass capilliary microelectrode are placed in contact with the protoplast .An electric field of low strength (10Kvm-1) gives rise to dielectrophoretic dipole generation within the protoplast suspension. This leads to pearl chain arrangement of protoplasts. Subsequent application of high strength of electric fields (100 kvm-1) for some microseconds results in electric breakdown of membrane and subsequent fusion Groth DI(1987) et al carried out electofusion of Penicillium protoplasts,after

diaelectrophoresis and found viable fusion products.

**Biotechnological applications of protoplast fusion**:

Protoplasts contained all the intracellular organelles of cells and form a vital link in transfer of micromolecules between cyto organelles, currently most of the laboratories engaging in fungal genetics are using gene manipulation procedures based on protoplasts. Therefore to further improve the genetic properties of these strains using protoplast fusion are attempt to develop methods for preparation and regeneration of protoplasts. The process involves protoplast mutagenesis, transformation and protoplast fusion. Fusants of Penicillium chrysogenum and Cephalosporium acremonium produced a novel lactam antibiotic . In 1989 protoplast fusion between two strains of Aspergillus niger 8-2 a fast growing strain and poor producer of glucoamylase and Aspergillus niger 8-7 ,a slow rowing strain and good producer of enzyme and the resulting fusant produced 68% more glucoamylase than parental strains .

This process is helpful in the production of a complete set of cellulases by the protoplast fusion of *Trichodermma reesei* and *A.niger* (one produced more amount of endo and exoglucanase and other produced more β- glucosidase .

**Genetic modification of an animal** involves altering its genetic material by adding, changing or removing certain DNA sequences in a way that does not occur naturally. It aims to modify specific characteristics of an animal or introduce a new trait, such as disease resistance or enhanced growth. DNA is the genetic material of an organism and carries the instructions for all the characteristics that an organism inherits. Changes introduced in an animal's genetic make-up can therefore be transmitted to the next generation.

While this technology has so far been used in plants for agriculture and in micro-organisms to produce enzymes, the potential application of genetic modification techniques to animals is also being researched. Several international organisations, including FAO/WHO and the United States Food and Drug Administration, have already published guidelines for the safety assessment of these animals and their derived products.

Animal biotechnology and its application in veterinary medicine and pharmaceuticals as well as improvement in food production. Knowledge of animal genetics is important in the application of biotechnology to manage genetic disorders and improve animal breeding. Genomics, proteomics and bioinformatics are also being applied to animal biotechnology. Transgenic technologies are used for improving milk production and the meat in farm animals as well as for creating models of human diseases. Transgenic animals are used for the production of proteins for human medical use. Biotechnology is applied to facilitate xenotransplantation from animals to humans. Genetic engineering is done in farm animals and nuclear transfer technology has become an important and preferred method for cloning animals.

Biotechnology has potential applications in the management of several animal diseases such as foot-and mouth disease, classical swine fever, avian flu and bovine spongiform encephalopathy. The most important biotechnology-based products consist of vaccines, particularly genetically engineered or DNA vaccines.

Gene therapy for diseases of pet animals is a fast developing area because many of the technologies used in clinical trials humans were developed in animals and many of the diseases of cats and dogs are similar to those in humans. RNA interference technology is now being applied for research in veterinary medicine Molecular diagnosis is assuming an important place in veterinary practice. Polymerase chain reaction and its modifications are considered to be important. Fluorescent in situ hybridization and enzyme-linked immunosorbent assays are also widely used.

In a potential new step for genetically modified food, babies could someday drink human-like milk derived from herds of genetically modified dairy cows, which scientists say could supplement breast milk and replace baby formula.

Scientists have created 300 cows that produce milk with some of the properties of human breast milk, including lysozyme, which fights bacteria and improves infants' immune systems in their first few days of life.

Researchers in China introduced genes that express human lysozyme (also called HLZ) and other human proteins into Holstein cattle embryos, and implanted the

embryos into surrogate cows. When the GM cows started lactating, their milk contained HLZ and two other proteins.

Human breast milk contains all the nutrients an infant needs, but cow milk is not as readily digested or absorbed. Making cow milk more human could give dairy products a nutritional boost . Despite the benefits that HLZ provides to breast-fed infants, mothers do not always desire to lactate and sometimes situations prevent lactation; therefore, the development of alternate sources of HLZ would be beneficial to infant health

The researchers believe human milk from cows could be a better breast milk alternative than baby formula.

## Gene therapy

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases. Many diseases such as ADA-SCID, X-linked SCID, Leber's congenital amaurosis (a retinal disease), Parkinson's disease, multiple myeloma, chronic and acute lymphocytic leukemia, adrenoleukodystrophy have reported of successful clinical trials. But these are still not approved by FDA. Some other diseases on which gene therapy based research is going on are Haemophilia, Tyrosinemia, Cystic Fibrosis and many other cancers.

## Types of gene therapy

There are several approaches for correcting faulty genes; the most common being the insertion of a normal gene into a specific location within the genome to replace a non functional gene. Gene therapy is classified into the following two types:

1. Somatic gene therapy

2. Germ line gene therapy

1 **Somatic Gene Therapy** In somatic gene therapy, the somatic cells of a patient are targeted for foreign gene transfer. In this case the effects caused by the foreign gene is restricted to the individual patient only, and not inherited by the patient's offspring or later generations.

**.2 . Germ Line Gene Therapy**

Here, the functional genes, which are to be integrated into the genomes, are inserted in the germ cells, i.e., sperm or eggs. Targeting of germ cells makes the therapy heritable.

## Methods of gene therapy

There are mainly two approaches for the transfer of genes in gene therapy:

1. Transfer of genes into patient cells outside the body (ex vivo gene therapy)

2. Transfer of genes directly to cells inside the body (in vivo)

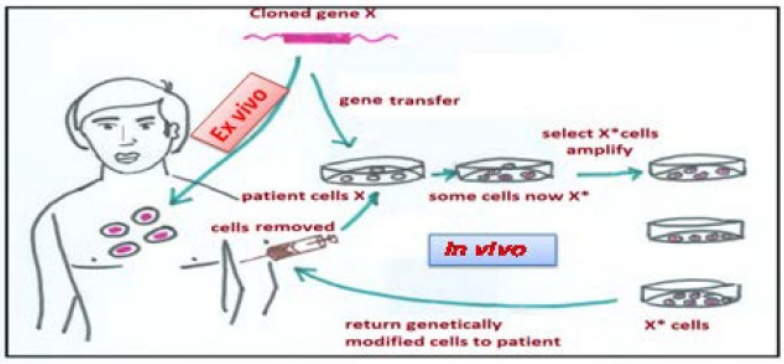



**Figure 8-15 Gene Therapy using autologous cells: Cells are used, i.e. cells are removed from the patient, cultured *in vitro*, before being returned to the patient's body. In this figure *in vivo* and *ex vivo* gene therapy is diagrammatically explained.**

## *Ex vivo* gene therapy

- In this mode of gene therapy genes are transferred to the cells grown in culture, transformed cells are selected, multiplied and then introduced into the patient.

- The use of autologous cells avoids immune system rejection of the introduced cells.

➢ The cells are sourced initially from the patient to be treated and grown in culture before being reintroduced into the same individual.

➢ his approach can be applied to the tissues like hematopoietic cells and skin cells which can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.

➢ Figure 8-1.5.1 shows a self explanatory schematic diagram for ex vivo gene transfer.

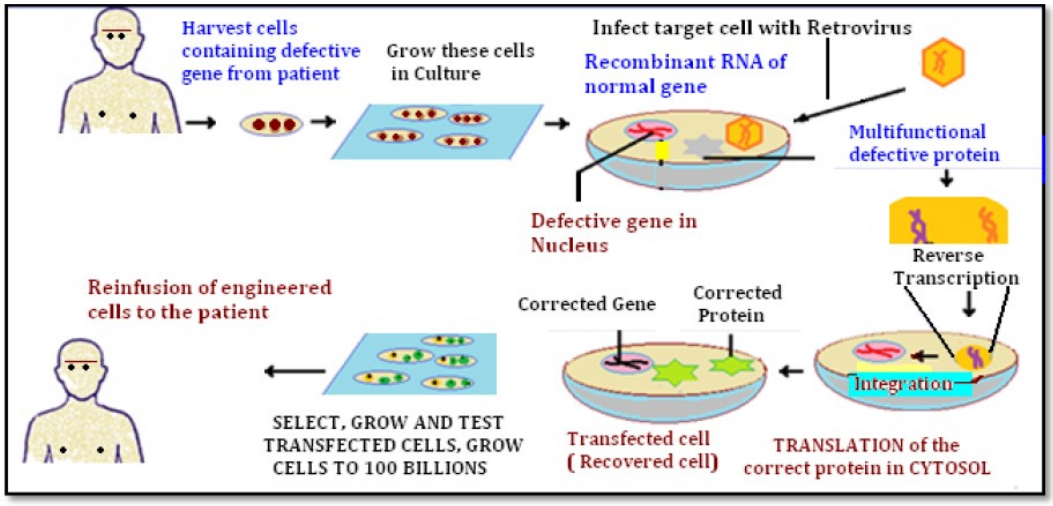



**Figure 8-1.5.1 *Ex vivo* therapy involves tightly regulated cellular manipulation in harvested cells**

**In vivo** method of gene transfer involves the transfer of cloned genes directly into the tissues of the patient.

➢ This is done in case of tissues whose individual cells cannot be cultured in vitro in sufficient numbers (like brain cells) and/or where re-implantation of the cultured cells in the patient is not efficient.

➢ Liposomes and certain viral vectors are employed for this purpose because of lack of any other mode of selection.

➢ In case of viral vectors such type of cultured cells were often used which have been infected with the recombinant retrovirus in vitro to produce modified viral vectors regularly. These cultured cells will be called as vector-producing cells (VPCs)). The VPCs transfer the gene to surrounding disease cells.

➢ The efficiency of gene transfer and expression determines the success of this approach, because of the lack of any way for selection and amplification of cells which take up and express the foreign gene.

➢ Figure 8-1.5.2 shows various steps of in vivo gene transfer.

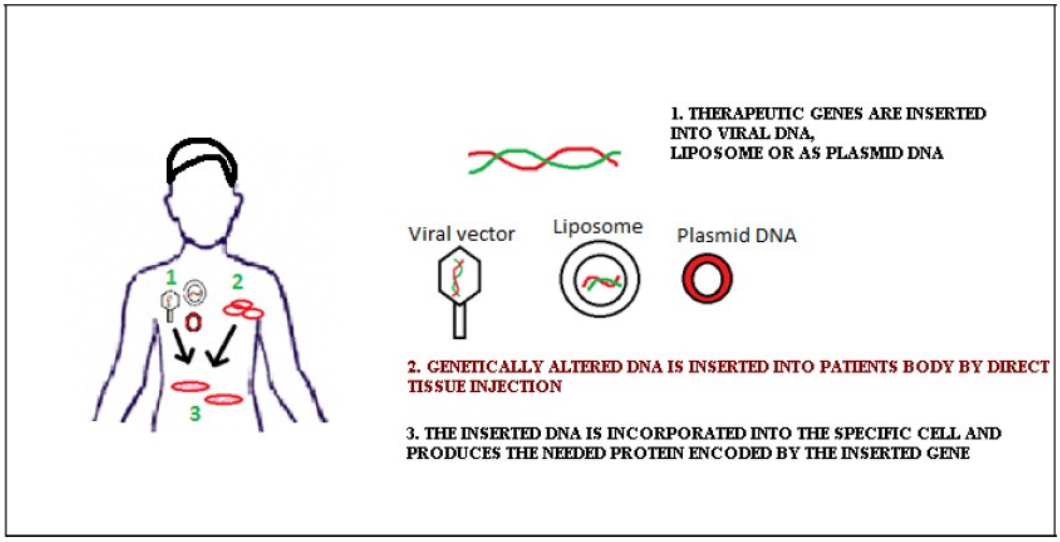



**Figure 8-1.5.2 various steps of *in vivo* gene transfer**

| Difference Between *in vivo* and *ex vivo* Gene Delivery Systems | |
|---|---|
| ***In vivo*** | ***Ex vivo*** |
| Less invasive | More invasive |
| Technically simple | Technically complex |
| Vectors introduced directly | No vectors introduced directly |
| Safety check not possible | Safety check possible |
| Decreased control over target cells | Close control possible |