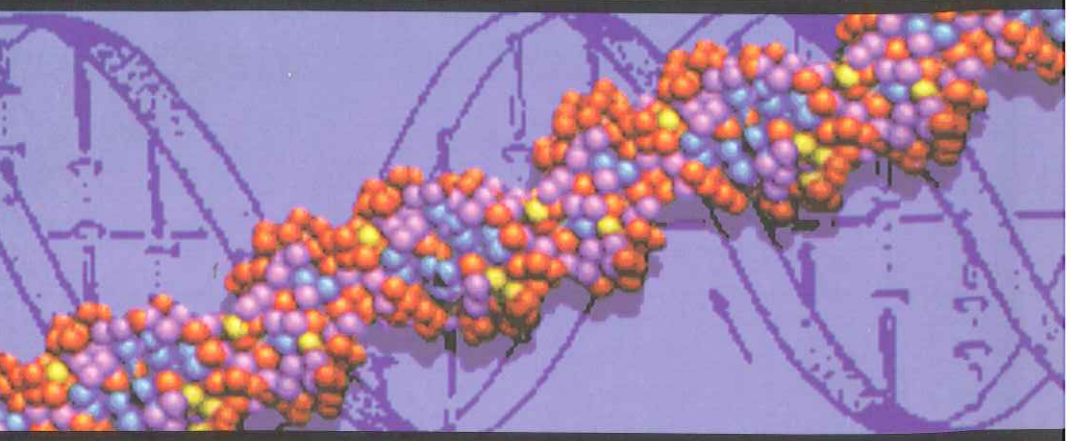
Biotechnology – Simplified



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A Publication by

ERA CONSUMER MALAYSIA

Education and Research Association For Consumers, Malaysia

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by Katherine Anne Francis

Contents

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ERA CONSUMER MALAYSIA

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(Education and Research Association for Consumers, Malaysia]

ERA CONSUMER is a voluntary, non-political and non-profit organization. ERA focuses on issues ranging from food security, human rights, environment and consumer rights to women's rights for a socially just and equitable society.

Foreword

Man has since the early days of his practising the planting of food crops and the breeding of animals for milk, meat and as beasts of burden, has been carrying out selective breeding of these plants and animals to serve him for his benefit.

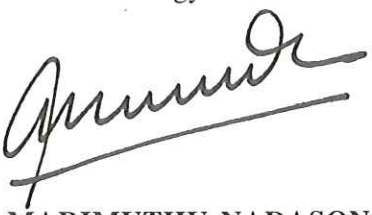
In modern times, man has used his knowledge of science in all areas of human endeavour, from food production to space travel and the unravelling of the mysteries of life as it was on Earth millions of years ago.

In recent times, there have been great advances in the use of technologies for food production and livestock breeding. Out of these advances have arisen concerns and, in the area of genetic engineering at least, the question is whether man is trying to play God is often asked.

Biotechnology is still a new science and many people still do not understand it. Our aim in producing this publication is to explain the subject of biotechnology in as simple a language as possible.

Since the early days and more so at present, biotechnology is dogged by controversies. In fact, every other month there claims and counter-claims on the pros and cons of biotechnology, followed often by rows between and among scientists, government agencies, watchdog groups, multinational corporations funding biotechnology research and concerned members of the civil society.

We do not wish to enter into the debates raging over this subject in this publication, which aims to just inform the reader about what biotechnology is all about. The controversies, the pros and cons and the generally accepted arguments on the impact of biotechnology on humankind will be taken up in another publication.



MARIMUTHU NADASON
President
ERA Consumer Malaysia

Glossary

Agrobacterium — A natural bacterium that can be used to transfer DNA genes into broadleaf plants, such as tobacco, tomato or soybean.

Chromosome — A cellular structure comprised of a long, folded DNA molecule and protein.

DNA — Deoxyribonucleic acid, the substance within cells that carries the "recipe" for the organism and is inherited by offspring from parents. The process by which genetic information is transferred is very complex. One of the nucleic acids, deoxyribonucleic acid, serves as the coding material. The genetic code is the relationship between the nucleotide sequence in nucleic acids and the amino acid sequence in proteins. As a result, the information for the structure and function of all living things is passed from one generation to the next. Scientists say that the workings of the genetic code are still far from being properly understood. (*see also* **RNA**)

DNA fingerprinting — The process of cutting a DNA chromosome with restriction enzymes and separating the pieces by electrophoresis to generate a unique pattern, the "fingerprint" for each species, breed, hybrid, or individual, depending on which enzymes and probes are used.

Electrophoresis — A laboratory technique for determining DNA fragment sizes by separating them in a gel placed in an electric field.

Electroporation — The use of an electric shock to transfer DNA into the cells of an organism; one of several procedures called transformation.

Eukaryote — Literally means "true nucleus". Eukaryotes are more complex organisms and can be single-celled or multicellular. A well-defined nucleus, set off from the rest of the cell by a membrane, is one of the chief features that distinguishes a eukaryote from a prokaryote.

Both **prokaryotic** and eukaryotic cells contain DNA. The total DNA of a cell is called the **genome**. A gene is an individual unit of heredity. It controls individual traits by coding for a functional protein or **RNA**.

A cell carries in its DNA the instructions for making the complete organism. When the cell divides, each new cell bears a copy of the original DNA. Replication of the hereditary material is made possible by the complementary nature of the DNA bases. Thus, one strand of DNA is the template for the other.

Starting in the 1970s, techniques have been introduced for manipulating the DNA by cutting and splicing it a manner that both mimics and transcends natural processes. These techniques provide valuable insight into the manner in which proteins interact with DNA molecules to control gene activation and repression.

Gene A functional unit of the DNA, one "word" in the DNA recipe.

Genetic code The information contained in DNA molecules that scientists describe on the basis of a 4-letter alphabet (A, C, G, and T).

Genetic engineering The process of transferring DNA from one organism into another that results in a genetic modification; the production of a transgenic organism.

Genetic map The locations of specific genes along a chromosome marked with probes.

Genome The entire DNA "recipe" for an organism, found in every cell of that organism.

Living Cells Living cells as they exist today are assemblages that include very large molecules, such as proteins, nucleic acids and polysaccharides. These are many times larger than the smaller molecules from which they are built.

Hundreds of thousands of these smaller molecules or **monomers** can be linked to form macromolecules, which are also called **polymers.** Carbon is able to form bonds with itself and with many other elements, giving rise to different kinds of monomers such as amino acids, nucleotides and monosaccharides (sugar monomers).

In present day cells, amino acids (the monomers) combine by polymerisation to form proteins and nucleotides (also monomers) combine to give nucleic acids. The polymerisation of sugar monomers produces polysaccharides.

Several classes of molecules play a key role in the life process. Among the most important are proteins and nucleic acids, both of which are polymers or very large molecules formed by linking together smaller units called monomers. In the case of proteins, the monomers are amino acids and in nucleic acids, the monomers are the nucleotides.

Mutation A change of one of the "letters" in the DNA "recipe", caused by chemicals, ultraviolet light, X-rays, or natural processes.

Organelle	A membrane-bounded portion of a cell with a specific function.
Particle gun	A gun that shoots DNA into the cells of an organism; the most versatile of a series of procedures called transformation.
PCR	Polymerase chain reaction, which rapidly duplicates specific DNA molecules in response to temperature changes in a computer-controlled heater.
Plasmid	A small, circular DNA that is used to transfer genes from one organism into another.
Pluripotent	Capable of giving rise to most tissues of an organism.
Probe	A very short piece of DNA used to find a specific sequence of "letters" in a very long piece of DNA from a chromosome or genome.
Prokaryote	Literally means "before the nucleus". Prokaryotes are single-celled organisms without a well-defined nucleus. Groups of them can exist in association, forming colonies with some differentiation of cellular functions.
Recombinant DNA	DNA formed by joining pieces of DNA from two or more organisms.
RNA	The discovery of the ribonucleic acid or RNA has profoundly affected the scientific debate on the origin of life on Earth. The RNA is a nucleic acid that is capable of catalysing its own further processing. Until this discovery, catalytic activity was associated exclusively with proteins. Today, many scientists believe that it is the RNA, rather than the DNA, that is the original coding material.
RFLP	Restriction fragment length polymorphism, which describes the patterns of different (polymorphism) sizes of DNA (fragment length) that result from cutting with restriction enzymes (restriction). See DNA fingerprinting above.
Sequence	The order of "letters" in the DNA "recipe." The DNA sequence is the chemical structure that contains information.
Somatic cell	Cell of the body other than egg or sperm.
Somatic cell nuclear transfer	The transfer of a cell nucleus from a somatic cell into an egg from which the nucleus has been removed.

Stem cells	Cells that have the ability to divide for indefinite periods in culture and to give rise to specialised cells.
Totipotent	Having unlimited capability. Totipotent cells have the capacity to specialise into extra-embryonic membranes and tissues, the embryo, and all post-embryonic tissues and organs.
Transformation	A procedure to transfer DNA into the cells of an organism. This can be done with Agrobacterium (most dicots), calcium chloride (bacteria), electroporation (any organism), or the particle gun (any organism).
Transgenic	An organism that has been modified by genetic engineering to contain DNA from another species.
Vector	Any vehicle or structure that is used to transfer DNA into an organism. The most commonly used are plasmid DNA vectors or viruses.

Biotechnology defined

Biotechnology is generally accepted to mean all technologies that manipulate the genes of all life forms, plant or animal. It can also be broadly defined as "using living organisms or their products for commercial purposes".

Break "biotechnology" into its root words and you have "bio", which means the use of biological processes, and "technology", which means to solve problems or make useful products.

Using biological processes is hardly a noteworthy event. Man began growing crops and raising animals in the Stone Age to provide a stable supply of food and clothing. We have used the biological processes of microorganisms for 6,000 years to make useful food products, such as bread and cheese, and to preserve dairy products and crops.

Since the early days of man, the practice of plant and animal domestication and breeding has been a crucial part of his survival. Till today, these practices, continued by small farmers and indigenous communities, have ensured the continued diversity of food crops and livestock.

A narrower and more specific definition of biotechnology is "the commercial application of living organisms or their products, which involves the deliberate manipulation of their genes".

This definition implies a set of laboratory techniques developed within the last 20 years that have been responsible for the tremendous scientific and commercial interest in biotechnology, the founding of many new companies and the redirection of research efforts and financial resources among established companies and universities.

These laboratory techniques have provided scientists with a spectacular vision of the design and function of living organisms, and have also provided technologists in many fields with the tools to implement new and innovative commercial applications.

A more appropriate definition of biotechnology today may well be "new

biotechnology", since it involves the use of the cellular and molecular processes to solve problems or make products. Biotechnology is a collection of technologies and what these technologies have in common is the use of cells and biological molecules.

Biotechnology is receiving so much attention today because of the huge profits it earns for those involved – scientists, researchers and of course, the manufacturers, specifically those conglomerates that market products resulting from the research and development work of scientists in their employ, and the global laws that allow these companies to patent anything that they claim is their "discovery".

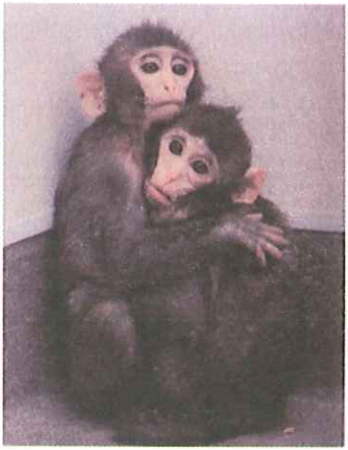


Fig. 1: Dolly, the most famous sheep in the world, produced by cloning techniques. Two rhesus monkeys, also the result of cloning.

During the 1960s and 70s, man's understanding of biology reached a point where we could begin to use the smallest parts of organisms, their cells and molecules, in addition to using whole organisms. The biological molecules we most often use are nucleic acids, such as DNA, and proteins.

All living organisms are composed of cells that contain a substance called deoxyribonucleic acid or DNA in their chromosomes. The structure of the DNA molecules in an organism contains information that is used by the cells as a "recipe" for that organism.

This means that the traits or characteristics of a living thing are essentially determined by the information in its DNA. The "words" for the DNA recipe, called genes, are derived from a 4-letter alphabet (A, C, G and T), and usually contain between 1,000 and 100,000 letters.

The entire recipe, called the genome, may contain between four million letters (for a simple bacteria) and three billion letters or more in the case of the human being.

Except for the sequence and number of letters in each recipe, DNA from any organism is chemically and physically the same. One of the most popular scientific claims of biotechnology is that DNA from any organism will function if transferred into any other organism. This claim has been hotly challenged and today scientists acknowledge that the random insertion of foreign genes into the genetic material of an organism may cause unexpected changes in the functioning of the other genes. Existing molecules may be manufactured in incorrect quantities, at the wrong times, or, new molecules may be produced.

If people are confused today because of this term "biotechnology", it may be because several other related terms have been associated or used together with it. Among the terms freely used are "genetic engineering" and "cloning".

Genetic engineering refers to all technologies that artificially move genes from one organism to another, often from one species to another (even from plant to animal and vice-versa), to produce "new" or "novel" organisms.

Genetic engineering is highly sophisticated laboratory work to manipulate the genetic material and other biologically important chemicals to change, modify or alter the DNA, the chemical in the cells of plants, animals and insects that stores their genetic code of existence.

While scientists have explained genetic engineering as "speeded-up selective breeding", institutions supporting biodiversity point out that these two are worlds apart.

Breeding does not manipulate genes. It involves the crossing of selected parents of the same or closely related species. In contrast, genetic engineering involves extracting selected genes from one organism (such as animals, plants, insects, bacteria or viruses or any two of these), or synthesising copies of them, and artificially inserting them into another completely different organism, such as a commercially important food crop.

Not all biotechnology is genetic engineering. Cloning is another type of genetic engineering, where an exact copy of an organism is produced through the direct manipulation of its DNA.

In genetic engineering, the genes of an organism are manipulated, modified or altered through the direct transfer of some or all of its traits to another organism through a process that can be called "splicing", or to use the term made common with the advent of computer word processing, the "cut and paste" method.

Usually, only one gene is transferred at a time, but genes from several donor organisms can be put into a single recipient. Therefore, by "cutting and pasting" DNA, scientists genetically engineer the transfer of genes from one type of organism into another type of organism (or species) on Earth.

Usually, a virus gene, or sometimes a bacteria gene, is used to "smuggle in" and promote the inserted gene in the host organism. Antibiotic resistant genes are used to act as markers. The inserted genes will be present in every cell of the host plant or animal.

If the donor organism and the recipient organism are from different species, the resulting genetically-engineered organism is called "transgenic". Genetic engineering has allowed scientists to move genes across natural boundaries, such as from man to pig, and vice-versa.

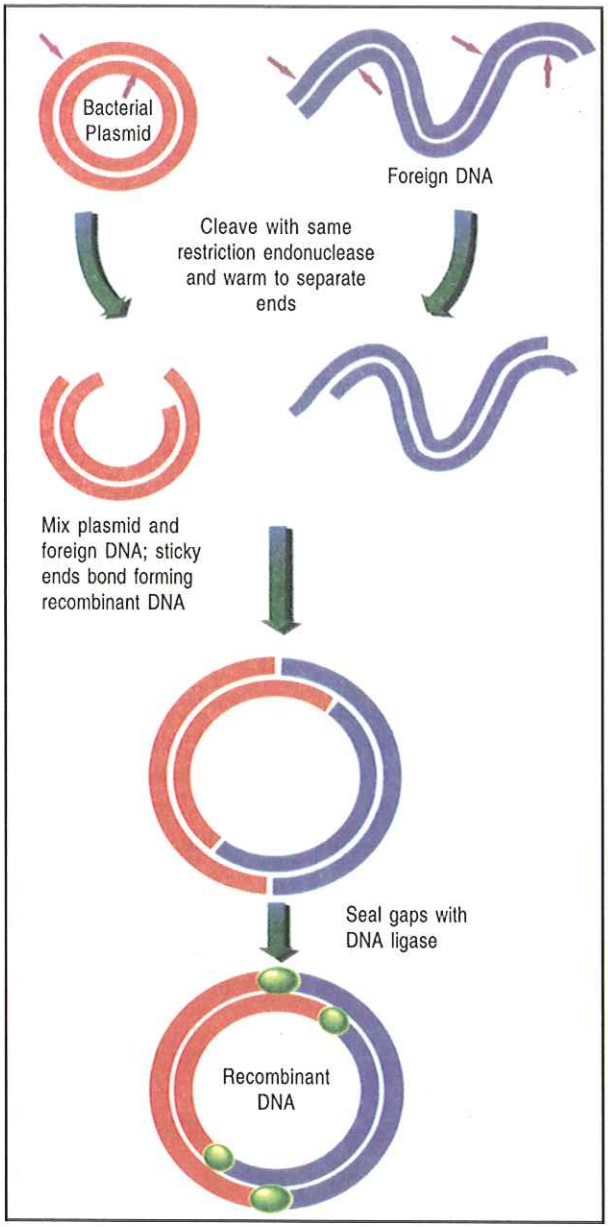


Fig 2 : The methodology for producing recombinant DNA.
Taken from Biochemistry. Third Edition (Mary K. Campbell, Mount Holyoke College); Saunders College Publishing, Harcourt Brace, College Publishers)

In transgenic engineering, the resulting organism can have new combinations of genes – and therefore new combinations of traits – that are not found in nature. The process of disarranging and recombining gene fragments of unrelated organisms (or species) in order to design a new organism, or even to create a new species is also known as "recombinant DNA technology".

Early days of biotechnology

Biotechnology seems to be leading a sudden new biological revolution. It has brought us to the brink of a world of "engineered" products.

Biotechnology has been described as "Janus-faced", implying that there are two sides to it.

On one, techniques allow DNA to be manipulated to move genes from one organism to another. On the other, it involves relatively new technologies whose consequences are untested – and therefore, should be met with caution.

The term "biotechnology" was coined by Karl Ereky, a Hungarian engineer, in 1919. At that time, the term meant all the lines of work by which products are produced from raw materials with the aid of living organisms. Ereky envisioned a Biochemical Age, similar to the Stone and Iron ages.

A common misconception is the thought that biotechnology only includes DNA and genetic engineering.

Biotechnology is not new. As we mentioned earlier, man has been manipulating living things to solve problems and improve his way of life for millennia. Early agriculture concentrated on producing food. Plants and animals were selectively bred and microorganisms were used to make food items such as beverages, cheese and bread.

The late 18th century and the beginning of the 19th century saw the advent of vaccinations, crop rotation involving leguminous crops and animal-drawn machinery.

The close of the 19th century was a milestone for biology. Microorganisms were discovered, Mendel's work on genetics was accomplished, and institutes for investigating fermentation and other microbial processes were established by Koch, Pasteur, and Lister.

Biotechnology at the beginning of the 20th century began to bring industry and agriculture together. During World War I, fermentation processes were developed

that produced acetone from starch, and paint solvents, for the rapidly growing automobile industry. Work in the 1930s was geared toward using surplus agricultural products to supply the industry, instead of imports or petrochemicals.

The advent of World War II brought about the manufacture of penicillin. The biotechnical focus moved to pharmaceuticals. The "Cold War" years were dominated by work with microorganisms in preparation for biological warfare as well as for antibiotics and fermentation processes.

Biotechnology and its applications

Cells and molecules are extraordinarily specific in their interactions. Because of this specificity, the tools and techniques of biotechnology believed to be quite precise. Scientists, through their manipulations, tailor the cells and molecules their isolate to operate in known, and predictable ways.

The aim is for the products of biotechnology to be better targeted to solving specific problems, generate lesser side effects and have fewer "unintended consequences". "Specific, precise and predictable" are the words big business and the proponents of biotechnology describe their world today, but many other scientists have challenged this notion. Modern genetics has shown that genes do not operate in isolation.

Although a gene can be cut out precisely from the DNA of an organism, its insertion into the DNA of another organism is entirely random. This results in the disruption of the order of genes in the chromosome of the host organism and may result in random and unexpected changes in the functioning of its cells.

Dr Richard Lewontin, Professor of Genetics at Harvard University, USA, has said of genetic engineering: "We have such a miserably poor understanding of how the organism develops from its DNA that I would be surprised if we do not get one rude shock after another."

The following are a few of the new biotechnologies that use cells and biological molecules, with examples of their applications in medicine, agriculture and environmental management provided:

Monoclonal antibody technology uses a type of immune system cell that makes proteins called antibodies. The antibodies we make to fight off a flu virus one winter can do nothing to protect us from a slightly different flu virus the next year. The specificity of antibodies makes them powerful tools for locating substances that occur in minuscule amounts and measuring them with great accuracy. For example, we use monoclonal antibodies to distinguish cancer cells from normal cells, and to:

- locate environmental pollutants
- detect harmful microorganisms in food
- diagnose infectious diseases in humans, animals and plants quicker and more accurately than ever before

Cell culture technology is the growing of cells outside of living organisms. Biotechnological control of insect pests is done by using insect cell culture to grow microorganisms that infect insect pests like mosquitoes and corn ear worms and kill them. It has been claimed that because of their specificity, biocontrol agents will infect problem insects without harming beneficial insects such as honeybees and ladybird beetles, but this contention by the proponents of genetic engineering has been disputed.

Mammalian cell culture often allows us to replace animal testing with cell testing when evaluating the safety and efficacy of medicines. In the future, we may be able to treat certain diseases, like diabetes and muscular dystrophy, by replacing malfunctioning cells with normal cells grown in culture.

At present, plant cell culture to produce naturally occurring compounds that have therapeutic value, such as the chemotherapeutic agent taxol, a compound found in yew trees, is being carried out.

Biosensor technology combines our knowledge of biology with advances in microelectronics. A biosensor is composed of a biological component, such as a cell or antibody, linked to a tiny transducer. Biosensors are detecting devices that rely on the specificity of cells and molecules to identify and measure substances at extremely low concentrations. When the substance of interest collides with the biological component, the transducer produces a digital electronic signal that will show the concentration of that substance.

Biosensors can

- measure the nutritional value, freshness and safety of food
- provide emergency room physicians with bedside measures of vital blood components
- locate and measure environmental pollutants
- measure blood glucose levels more rapidly and precisely than existing methods

Genetic modification technology, often referred to as **recombinant DNA biotechnology** is made, both in nature and by humans, particularly farmers and livestock breeders, by combining genetic material from two different sources. Humans began to preferentially combine the genetic material of domesticated plants and animals thousands of years ago by selecting the specimen for reproduction. Certain organisms had traits mankind valued, so these were chosen as parents for the next generation.

By selectively breeding individuals with valuable genetic traits and excluding others from reproduction, man intentionally changed the genetic make-up of the plants and animals that were domesticated.

Techniques for making selective breeding more predictable have been continually evolving, especially since the genetic basis of heredity was discovered in the early1900s. DNA molecules containing covalently linked segments derived from two or more DNA sources are called recombinant DNA.

Another name for **recombinant DNA** is **chimeric DNA,** named after chimera, a monster in Greek mythology that had the head of a lion, the body of a goat and the tail of a serpent. Producing recombinant DNA requires cutting and splicing two strands of DNA molecules in very specific ways.

Single genes, whose functions are known, are moved from one organism to another. In selective breeding, large sets of genes of unknown function are transferred. By making manipulations more precise and the outcomes more certain, scientists decrease the risk of producing organisms with unexpected traits and avoid the time-consuming trial-and-error approach of selective breeding.

This issue has aroused public interest and concern and has influenced medicine,

industry, agriculture and environmental problem-solving in the 20 years since its inception.

In medicine, faster and more efficient diagnosis and treatment of diseases such as cystic fibrosis, cancer, sickle cell anemia and diabetes are being developed. Recombinant organisms will be used in industry to produce new vaccines, solvents and chemicals of all kinds.

Now, in addition to using selective breeding to combine valuable genetic material from different organisms, we combine genes at the molecular level using the more precise techniques of genetic modification. Selective breeding and genetic modification fundamentally resemble each other, but there are important differences.

Currently, genetic modification is used to

- produce new vaccines
- treat some genetic diseases
- provide new medicines
- enhance biocontrol agents in agriculture
- develop biodegradable plastics

Antisense technology decreases the production of specific proteins by using small nucleic acids to block the genes responsible for making these proteins. Currently, researchers are investigating how to use this technology to

- slow down the spoilage of food
- control viral diseases
- inhibit inflammation
- treat diseases such as asthma, cancers and a kind of anaemia called thalassemia

Protein-engineering technology is often used in conjunction with genetic modification to improve existing proteins, usually enzymes, and to create proteins not found in nature. These new and improved proteins are meant to encourage the development of ecologically sustainable industrial processes because they are renewable, biodegradable resources.

Enzymes trigger the biochemical reactions on which all living organisms depend. Unlike most chemical catalysts used in industrial manufacturing processes, these biocatalysts dissolve in water and work best at neutral pH and comparatively low

temperatures. In addition, because biocatalysts are more specific than chemical catalysts, they produce fewer unwanted by-products.

The chemical, textile, pharmaceutical, pulp and paper, food and feed, metal and minerals and energy industries have all benefited from cleaner, more energy-efficient production made possible by incorporating biocatalysts into their production processes.

The traits that make biocatalysts environmentally advantageous may, however, become detrimental in certain industrial processes. Enzymes function in a narrow temperature range, and most fall apart at temperatures above 1,000° F. Scientists are circumventing these limitations by using protein engineering to increase enzyme stability under harsh manufacturing conditions.

What's new since 1972 is that scientists have been able to identify the specific DNA genes for many desirable traits and transfer those genes, usually carried on a plasmid or virus, into another organism. This is the process of genetic engineering, and the transfer of DNA is accomplished using either direct injection or the Agrobacterium, electroporation or particle gun transformation techniques.

These techniques provide the method to transfer DNA between any living cell, whether plant, animal, insect, bacterial or viral. Virtually any desirable trait found in nature can, in principle, be transferred into any chosen organism. An organism modified by genetic engineering is called transgenic.

Products of genetic engineering

Specific applications of genetic engineering are increasing rapidly in number. Genetic engineering is being used in the production of pharmaceuticals, in gene therapy and in the development of transgenic plants and animals.

1. **Pharmaceuticals:** Human drugs such as insulin for diabetics, growth hormone for individuals with pituitary dwarfism and tissue plasminogen activator for heart attack victims, as well as animal drugs like the growth hormones, bovine or porcine somatotropin, are being produced by the fermentation of transgenic bacteria that have received the appropriate human, cow or pig gene.
2. **Gene therapy:** The first clinical gene therapy is under way to correct an enzyme deficiency called ADA in children. In this treatment, bone marrow cells are removed, defective DNA in bone marrow cells is supplemented with a copy of normal DNA and the repaired cells are then returned to the patient's body to continue the repair process.

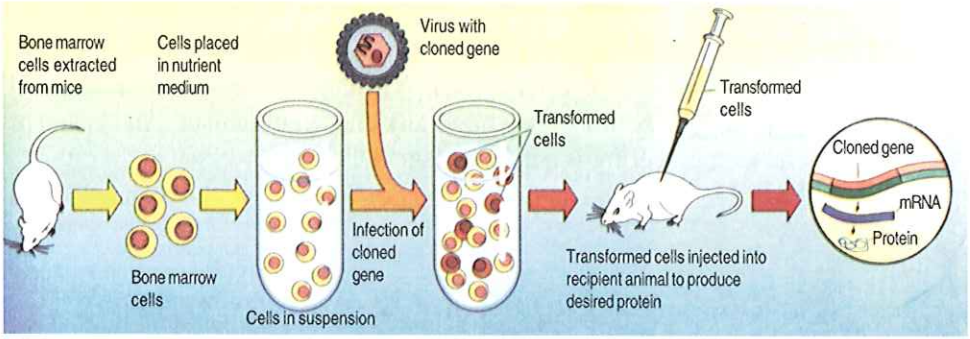


Fig 3 : Gene therapy in bone marrow cells. A cloned gene that directs the synthesis of a missing protein is introduced into bone marrow from the mouse. The transformed cells are replaced in the mouse's body, where they produce the desired protein.
Taken from Biochemistry. Third Edition (Mary K. Campbell, Mount Holyoke College); Saunders College Publishing, Harcourt Brace, College Publishers)

3. **Transgenic plants:** Transgenic plants that are more tolerant of herbicides, resistant to insect or viral pests or express modified versions of fruits or flowers have been grown and tested in outdoor test plots since 1987. The genes for these traits have been delivered to the plants from other unrelated plants, bacteria or viruses by genetic engineering techniques.

4. **Transgenic animals:** At present, most transgenic animals are designed to assist researchers in the diagnosis and treatment of human diseases. Several companies have designed and are testing transgenic mammals that produce important pharmaceuticals in the animal's milk. Products such as insulin, growth hormone and tissue plasminogen activator that are currently produced by fermentation of transgenic bacteria may soon be obtained by milking transgenic cows, sheep or goats.

Biotechnology in diagnostic applications

Since each living creature is unique, each has a unique DNA recipe. Individuals within any given species, breed, or hybrid line can usually be identified by minor differences in their DNA sequences – as few as one difference in a million letters can be detected! Using the techniques of DNA fingerprinting and polymerase chain reaction (PCR), scientists can diagnose viral, bacterial or fungal infections, distinguish between closely related individuals, or map the locations of specific genes along the vast length of the DNA molecules in the cells.

Identifying organisms

DNA fingerprints can be generated through the use of *restriction fragment length polymorphism* (RFLP) technology. An individual organism can be uniquely identified by its DNA fingerprint. Consequently, this fingerprint can be used to determine family relationships in paternity litigation, match organ donors with recipients in transplant programmes, connect suspects with DNA evidence left at the scene of a crime (in the form of hair or body fluids) or serve as pedigree for seed or livestock breeds.

Identifying genes

One important aspect of genetic engineering projects is to identify the DNA gene that controls a particular trait. In the same way that a visitor might use the state, city, street and house number to locate a friend's house, genetic engineers use genetic "maps" to locate genes.

The genetic maps are generated by statistical analyses, PCR, RFLP and DNA sequencing. Maps are being developed for humans, mice, swine, cattle, corn, wheat

and other plants or animals with commercial or research importance.

Diagnosing infectious diseases and genetic disorders

The diagnosis of infectious diseases is a profound application of new DNA technology. Tuberculosis, AIDS, papillomavirus and many other infectious diseases, in addition to the inherited disorders like cystic fibrosis or sickle cell anaemia, are diagnosed within hours by the PCR technique, instead of the days or weeks this will take by traditional methods.

The greatly increased sensitivity and speed of the PCR technique, as compared with traditional methods, allows earlier intervention and treatment. PCR testing kits will soon be available to diagnose diseases of crops and livestock.

Genetic engineering in eukaryotes

In bacteria, altering the genetic make-up of a cell means changing the whole single-celled organism. In multicellular organisms, one possibility is to change a gene in a specific tissue, one that contains only one kind of differentiated cell. This change is called somatic, affecting only the body tissues of the altered organism.

In contrast, changes made to the egg and sperm cells (or germ cells), called germ-line modifications, are passed on to succeeding generations. Changes desired in a germ cell must be done at an early stage of its development, before the germ cells are sequestered from the rest of the organism.

Germ-line alterations in humans raise serious moral and ethical questions, but these have been done in mice and commercially important food crop plants and livestock.

In mice, the method involves the microinjection of DNA that carries the gene to be introduced into the nucleus of a fertilised mouse egg. The injected DNA integrates itself into the genome. Eggs treated in this manner are injected into a foster mother to develop.

When the mice are born, DNA samples are taken from the tails to test for the incorporation of the injected DNA.

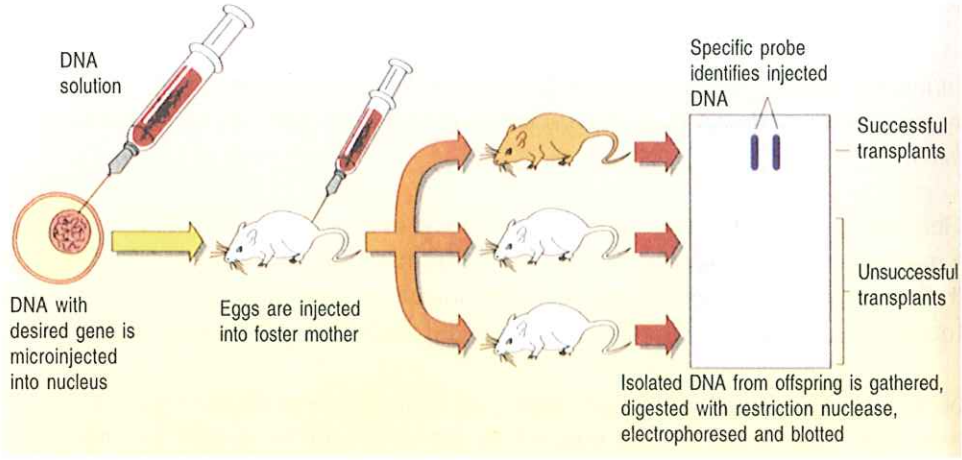


Fig 5: The procedure for producing transgenic mammals. The two mice on the bottom have not received new genes. The mouse on top has received a new gene, which it can pass on to its offspring. The mouse on the top is a transgenic organism and the altered gene is the transgene.
Taken from Biochemistry. Third Edition (Mary K. Campbell, Mount Holyoke College); Saunders College Publishing, Harcourt Brace, College Publishers)

The future of biotechnology

The structure of the DNA was deciphered by James Watson, a geneticist, and Francis Crick, a physicist, thus marking the beginning of molecular biology in the 20th century. Their determination of the physical structure of the DNA molecule became the foundation for modern biotechnology, enabling scientists to develop new tools to improve the future of mankind.

Biotechnology has applications in both plant and animal breeding. Scientists are developing disease- and herbicide-resistant crops, disease-resistant animals, seedless fruits and fast-growing chicken. Microbes are being engineered to digest compounds that are currently polluting the environment.

Some of the newer frontiers of biotechnology include protein-based "biochips", which may replace silicon chips. It is believed that biochips would be faster and more energy efficient.

Biochip implants in the body may be able to deliver precise amounts of drugs to affect heart rate and hormone secretion or to control artificial limbs. Biosensors are monitors that use enzymes, monoclonal antibodies or other proteins to test air and water quality, to detect hazardous substances and to monitor blood components *in vivo*.

Gene therapy involves the correction of defects in genetic material. In this process, a normal gene is introduced to replace a malfunctioning one. Gene therapy will be the "expression" of the medical research branch of biotechnology. It may in time form the basis for its own industry, or join the traditional pharmaceutical industry.

New delivery systems, called liposomes, are being developed to get cytotoxic drugs to tumor sites with minimal damage to the surrounding healthy tissues. New monoclonal antibodies will be isolated for use in cancer treatment, diagnostic testing, bone marrow transplant and other applications.

Whatever the future of these particular ventures, it seems molecular biology and biotechnology will be important sciences of the coming century.

The age-old practice of farmers to selectively breed plants and animals for food and other human activities is considered today to be a primitive form of genetic engineering. The farmers had to cope with changes that arose spontaneously, and the only choice was whether to breed for a trait or to let it die out.

All this has been changed with the understanding of the molecular nature of heredity and the ability to manipulate those molecules in the laboratory.

The practice of selective alteration of organisms for agricultural and medical purposes has profited greatly from recombinant DNA methods. Genes for increased yields, frost resistance, disease resistance and resistance to pests have been introduced into commercially important plants such as maize, rice, strawberries, tomatoes, potatoes, canola, soyabean and peanuts.

Similarly, animals of commercial importance and fish have also been genetically altered. Some variations introduced in animals have medical implications, such as mice with altered genetic make-up, which are used in research laboratories.

In another medical-related field, researchers working with insect-borne diseases

such malaria are trying to engineer strains of insects such as the *Anopheles gambiae* mosquito, which spreads malaria, so that it can no longer spread the disease to humans.

Recombinant DNA technology makes it possible to change specific genes and even specific DNA sequences within those genes in order to alter the inherited characteristics of an organism.

Bacteria can be altered to produce large amounts of medically and economically important proteins, animals can be treated to cure and alleviate diseases and agriculturally-important plants can be made to produce greater crop yields or be given increased resistance to pests.

However, many of these remain claims and are the result of a few controlled laboratory tests. The average scientist will still raise his doubts and point out to the many cases of field testing where altering the inherited characteristics of an organism have brought disastrous results.

Genomes of all organisms are subject to a host of destabilising processes that mutate, rearrange and alter DNA. Genes transferred to a particular part of an organism will have the increased likelihood of being transferred elsewhere, producing unpredictable results. These genes can also be transferred to another organism, or another species.

Plant tissue culture

In 1965, French botanist George Morel was attempting to obtain a virus-free orchid plant when he discovered that a millimetre-long shoot could be developed into complete plantlets by micropropagation. This was the beginning of tissue culture.

Thereafter, in the 1970s, developed countries began commercial exploitation of this technology. It entered the developing world in the 1980s. It was earlier used to develop ornamental plants and flowering plants for export.

With tree species, the technique of tissue culture remained confined for many years to the laboratory stage and had generally invited only academic interest. But in most developing countries, the shortage of biomass and the ever-increasing energy

requirements created the need to explore possibilities of mass propagation of trees by tissue culture.

Tissue culture or mass cloning methods of elite tree species is done for increasing land productivity. They are being modified or adapted for large-scale modification.

However, in Malaysia the use of cloning technology to produce better rubber trees has been put to use since the 1920s.

When the local farmers and farmers and horticulturists carried out their "cloning" of plants years and years ago, they of course did not use the term. They used terms like *kahwin* to mean bud grafting, growing plants from cuttings and even marcotting, all actually ways of cloning plants. Perhaps the most famous clone in Malaysia is the D24 durian!

Cloning as a way of making lots of copies of the best plants has been put into use for the production of better rubber trees in Malaysia since the 1920s. This is done using bud grafting, and has allowed us to make many copies of the best trees and produce much more latex than if we just grew all the trees from seed.

Tissue culture and the micropropagation of plants are some of the newer ways of making more copies of the best plants. Tissue culture means the method of growing plant or animal cells outside of the organism.

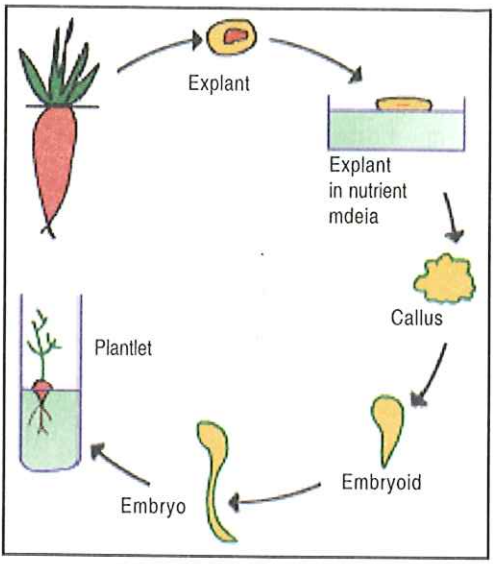
Also known as "in vitro propagation" or "micropropagation", plant tissue culture is usually done in sterile (microbe-free) conditions using liquid or jelly as a growth medium (in place of soil). It permits the manipulation of physical and chemical conditions in the production of large numbers of high quality plant material within a short period of time.

Many of our local bananas are now produced through tissue culture, which is case really just a faster way to grow banana plants in the laboratory.

There are other advantages, since tissue culture allows us to produce much more uniform plants in a clean environment. These plants will all fruit at the same time, are disease-free and, by selecting the best starting materials, will produce a better yield than if ordinary banana plants are used as planting materials.The same is true for other tissue-cultured planting materials.

Species are selected for tissue culture on the following basis:

- Species that have regeneration problems, especially because of poor seed set or germination (as in Anogeissus and bamboo). In these cases, seeds collected from superior trees are used for initiating cultures;
- Species that vary markedly in their desirable traits, i.e. Eucalyptus. The selected trees are marked from the variant population for the desirable trait such as disease resistance, straight bole, higher productivity, etc in consultation with officials from the state forest department or growers; and
- Species where plants of any one particular sex is of commercial importance, for example female plants of papaya and male plants of asparagus.



Tissue culture and totipotency

In tissue culture, the cells from the tissues and organs of a plant are separated. These separated cells are grown in containers with a nutrient media, under controlled conditions of temperature and light. The cultured plant requires a source of energy from sugar, salts, a few vitamins, amino acids, etc. that are provided in the nutrient media. From these cultured parts, an embryo or a shoot bud may develop, which then grows into a whole new plantlet.

Similarly, portions of organs or tissues can be cultured in a culture media. Generally, these give rise to an unorganised mass of cells called callus, which is the soft tissue that forms over a cut surface.

Tissue culture plantlets have poor photosynthesis efficiency and lack the proper mechanism to control water loss. They need to be hardened gradually by moving them along a humidity gradient in the greenhouse. Once these plants are in the research fields, they are evaluated under field conditions and the data is collected every six months. A large number of tissue culture plants that have grown into trees

are remarkably uniform and show an increase in biomass production over the conventionally raised plants.

Application of tissue culture

Micropropagation: This is the rapid vegetative multiplication of valuable plant material for agriculture, horticulture, and forestry.

Production of disease-free plants: When the apex of shoot is used for multiplication by tissue culture, we get disease-free plants because the shoot apical meristem, a group of dividing cells at the tip of a stem or root, is free from pathogens.

Plant breeding: Tissue culture has also been successfully used in plant breeding programmes.

Production of disease- and pest-resistant plants: Plants grown from tissue culture usually pass through the callus phase and show many variations. These show some agronomic characteristics like tolerance to pests, diseases, etc.

Cloning: This technique is used extensively in the commercial field for micropropagation of ornamental plants like chrysanthemum, gladiolus, etc. and also crops such as sugarcane, tapioca, and potato. Thus an unlimited number of plants that are genetically similar or are clones can be produced in a short span of time by tissue culture.

Large-scale propagation: To bridge the gap between research and application, the Department of Biotechnology, Government of India sponsored the setting-up of two pilot-scale facilities for large-scale propagation of elite planting material of forest trees through tissue culture. One of these facilities has been established on a 36-hectare site in Gual Pahari, Haryana, with an annual capacity of a million plantlets. Research at these facilities focuses exclusively on developing new protocols for mass cloning of elite planting material, mainly of trees.

The advantages of propagation by tissue culture include the elimination of diseases and the production of disease-free plantlets, the rapid production of large numbers of genetically identical plantlets, the introduction of new varieties and or genotypes, the preservation of germplasm, the production of haploid plants which can be used

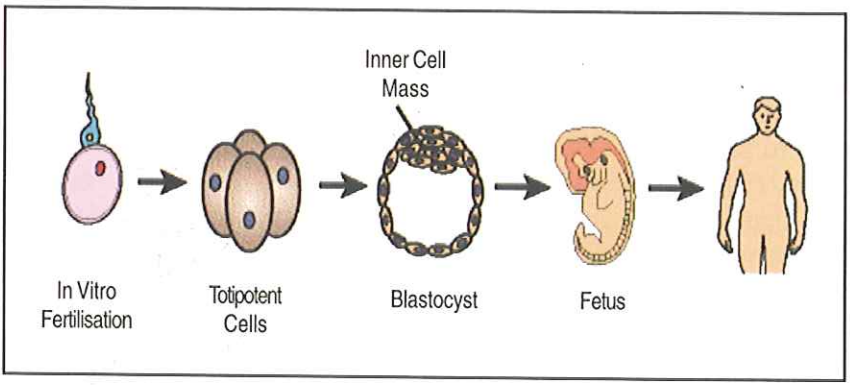
for plant breeding and the production of plantlets from species in which plant development from seed is difficult

Stem cells

Recent published reports on the isolation and successful culturing of the first human pluripotent stem cell lines have brought biomedical research to the edge of a new frontier. The development of these human pluripotent stem cell lines deserves close scientific examination, evaluation of the promise for new therapies, and prevention strategies, and open discussion of the ethical issues.

In order to understand the importance of this discovery as well as the related scientific, medical, and ethical issues, it is absolutely essential to first clarify the terms and definitions.

Stem cells have the ability to divide for indefinite periods in culture and to give rise to specialised cells. They are best described in the context of normal human development. Human development begins when a sperm fertilises an egg and creates a single cell that has the potential to form an entire organism.



This fertilised egg is called **totipotent**, meaning that its potential is total. In the first hours after fertilisation, this cell divides into identical totipotent cells. This means that either one of these cells, if placed into a woman's uterus, has the potential to develop into a foetus *(see Figure above).*

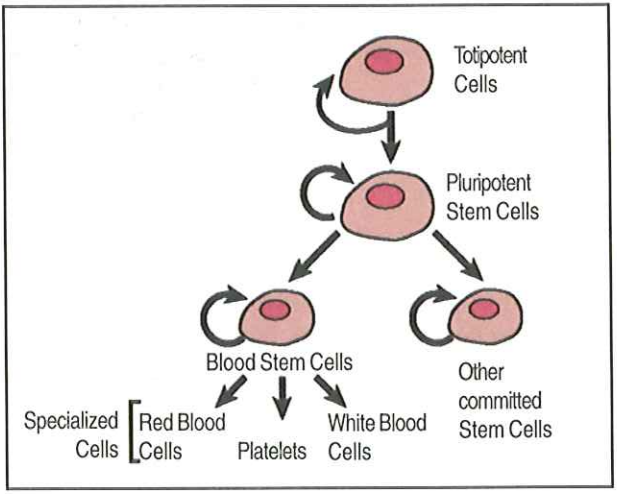
Identical twins develop when two totipotent cells separate and develop into two individual, genetically identical human beings. Approximately four days after fertilisation and after several cycles of cell division, these totipotent cells begin to specialise, forming a hollow sphere of cells, called a blastocyst. The blastocyst has an outer layer of cells and inside the hollow sphere, there is a cluster of cells called the inner cell mass.

The outer layer of cells will go on to form the placenta and other supporting tissues needed for foetal development in the uterus. The inner cell mass cells will go on to form virtually all of the tissues of the human body.

Although the inner cell mass cells can form virtually every type of cell found in the human body, they cannot form an organism because they are unable to give rise to the placenta and supporting tissues necessary for development in the human uterus.

These inner cell mass cells are **pluripotent** – they can give rise to many types of cells but not all types of cells necessary for foetal development. Because their potential is not total, they are not totipotent and they are not embryos. In fact, if an inner cell mass cell were placed into a woman's uterus, it would not develop into a fetus.

The pluripotent stem cells undergo further specialisation into stem cells that are committed to give rise to cells that have a particular function. Examples of this include blood stem cells that give rise to red blood cells, white blood cells and platelets; and skin stem cells that give rise to the various types of skin cells. These more specialised stem cells are called **multipotent** *(see Figure on the right).*



While stem cells are extraordinarily important in early human development, multipotent stem cells are also found in children and adults.

For example, consider one of the best understood stem cells, the blood stem cell. Blood stem cells reside in the bone marrow of every child and adult, and in fact, they can be found in very small numbers circulating in the blood stream. Blood stem cells perform the critical role of continually replenishing our supply of blood cells – red blood cells, white blood cells, and platelets – throughout life. A person cannot survive without blood stem cells.

The pluripotent stem cells undergo further specialisation into stem cells that are committed to give rise to cells that have a particular function, such as the blood stem cells that give rise to red blood cells, white blood cells and platelets. These more specialised stem cells are called **multipotent**.

The isolation of human pluripotent stem cells is important to science and to advances in health care. Pluripotent stem cells can help us to understand the complex events that occur during human development.

We will be able to understand the factors involved in the cellular decision-making process that result in cell specialisation. Turning genes on and off is key to this process, but little is know about these "decision-making" genes or what turns them on or off.

Some of the most serious medical conditions, such as cancer and birth defects, are the result of abnormal cell specialisation and cell division. A better understanding of normal cell processes will allow man to further delineate the fundamental errors that cause these often-deadly illnesses.

Human pluripotent stem cell research could also dramatically change the way we develop drugs and test them for safety. For example, new medications could be initially tested using human cell lines. Cell lines are currently used in this way (for example cancer cells). Pluripotent stem cells would allow testing in more cell types. This would not replace testing in whole animals and testing in human beings, but it would streamline the process of drug development. Only the drugs that are both safe and appear to have a beneficial effect in cell line testing would graduate to further testing in laboratory animals and human subjects.

Perhaps the most far-reaching potential application of human pluripotent stem cells is the generation of cells and tissue that could be used for so-called "cell therapies." Many diseases and disorders result from disruption of cellular function or destruction

of tissues of the body.

Today, donated organs and tissues are often used to replace ailing or destroyed tissue. Unfortunately, the number of people suffering from these disorders far outstrips the number of organs available for transplantation. Pluripotent stem cells, stimulated to develop into specialised cells, offer the possibility of a renewable source of replacement cells and tissue to treat a myriad of diseases, conditions, and disabilities, including Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis and rheumatoid arthritis.

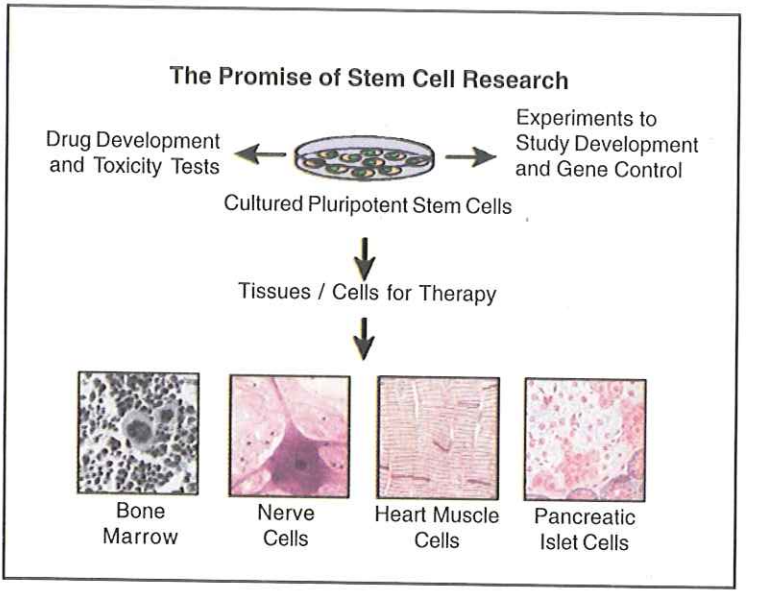
For example, the transplant of healthy heart muscle cells could provide new hope for patients with chronic heart disease whose hearts can no longer pump adequately. The hope is to develop heart muscle cells from human pluripotent stem cells and transplant them into the failing heart muscle in order to augment the function of the failing heart. Preliminary work in mice and other animals has demonstrated that healthy heart muscle cells transplanted into the heart successfully repopulate the heart tissue and work together with the host cells. These experiments show that this type of transplantation is feasible.

In the many individuals who suffer from Type I diabetes, the production of insulin by specialised pancreatic cells, called islet cells, is disrupted. There is evidence that transplantation of either the entire pancreas or isolated islet cells could mitigate the need for insulin injections. Islet cell lines derived from human pluripotent stem cells could be used for diabetes research and, ultimately, for transplantation.

While this research shows extraordinary promise, there is much to be done before we can realize these innovations. Technological challenges remain before these discoveries can be incorporated into clinical practice. These challenges, though significant, are not insurmountable.

Until recently, there was little evidence in mammals that multipotent cells such as blood stem cells could change course and produce skin cells, liver cells or any cell other than a blood stem cell or a specific type of blood cell.

However, research in animals has shown that some adult stem cells previously thought to be committed to the development of one line of specialised cells are able to develop into other types of specialised cells.



For example, recent experiments in mice suggest that when neural stem cells were placed into the bone marrow, they appeared to produce a variety of blood cell types. In addition, studies with rats have indicated that stem cells found in the bone marrow were able to produce liver cells. These exciting findings suggest that even after a stem cell has begun to specialise, the stem cell may, under certain conditions, be more flexible than first thought.

Research on human adult stem cells suggests that these multipotent cells have great potential for use in both research and in the development of cell therapies. For example, there would be many advantages to using adult stem cells for transplantation.

If adult stem cells can be isolated, "coaxed" into dividing and directing their specialisation and are then transplanted back into the patient, it is unlikely that such cells would be rejected. The use of adult stem cells for such cell therapies will certainly reduce or even end the practice of using stem cells derived from human embryos or human foetal tissue, which trouble many people on ethical grounds.

Any attempt to use stem cells from a patient's own body for treatment would require that stem cells would first have to be isolated from the patient and then grown in culture in sufficient numbers to obtain adequate quantities for treatment.

For some acute disorders, there may not be enough time to grow enough cells to use for treatment. In other disorders, caused by a genetic defect, the genetic error would likely be present in the patient's stem cells. Cells from such a patient may not be appropriate for transplantation.

There is evidence that stem cells from adults may have not have the same capacity to proliferate as younger cells do. In addition, adult stem cells may contain more DNA abnormalities, caused by exposure to daily living, including sunlight, toxins, and by expected errors made in DNA replication during the course of a lifetime. These potential weaknesses could limit the usefulness of adult stem cells.

Given the enormous promise of stem cells to the development of new therapies for the most devastating diseases, it is important to simultaneously pursue all lines of research. Science and scientists need to search for the very best sources of these cells. When they are identified, regardless of their sources, researchers will use them to pursue the development of new cell therapies.

The development of stem cell lines, both pluripotent and multipotent, that may produce many tissues of the human body is an important scientific breakthrough. It is not too unrealistic to say that this research has the potential to revolutionize the practice of medicine and improve the quality and length of life. (*Much of the background and graphics for this section on stem cells came from the National Institutes of Health (US) website).*

Versatility of the adult stem cell

University of Minnesota (US) scientist Dr Catherine Verfaillie and her team have now published peer-reviewed evidence that an adult bone marrow stem cell can proliferate extensively in culture and form virtually any tissue type – properties once claimed exclusively for embryonic stem cells.

Reporting on Dr Verfaillie's research on Jan 26, 2002, *New Scientist* said her team had discovered what might be "the most important cell ever", capable of producing any tissue in the body. "If so," the *New Scientist* continued "there would be no need to resort to therapeutic cloning."

These stem cells were dubbed "Multipotent Adult Progenitor Cells" (MAPCs). Since

then, the team has produced several reports on the MAPCs, including their isolation from rodents and humans, their growth abilities and their capacity to transform into various other tissues, including blood vessels, nerves and liver.

Dr Verfaillie's team found that MAPCs could be grown in culture over 120 generations – more than twice the number previously thought possible for adult stem cells – without losing their capacity to differentiate into other tissues.

The MAPCs could be directed in culture to form specific tissues from each of the three "primary germ layers", which are the three main developmental types of tissues – an indication that the cells can potentially form any tissue. When injected into mice, the cells participated in formation of various tissues, including blood and marrow, liver, lung, and gut.

The MAPCs thus show the potential of being able to do everything proposed clinically for cloning and embryonic stem cells, yet without tumour formation, and with the ability to use the patient's own cells to overcome the problem of immune rejection.

The researchers noted that since MAPCs proliferate extensively without obvious aging or loss of differentiation potential, "they may be an ideal cell source for therapy of inherited or degenerative diseases".

In what is considered the "gold standard" test for pluripotency (i.e. the ability to form any adult tissue) Dr Verfaillie and her team put the adult bone marrow stem cells to the same test used to prove the pluripotency of mouse embryonic stem cells. The result showed that single MAPC cells injected into early mouse embryos could form most, if not all, of the tissues of the developing mice. (Adapted from *Do No Harm: The Coalition of Americans for Research Ethics – website: www.stemcellresearch.org*).

Adult skin cells reprogrammed without cloning

A team of scientists from Norway has succeeded in coaxing one type of adult cell to start behaving like a completely different type of adult cell. The scientists have made human skin cells in a test tube behave as if they were immune system cells, by bathing the skin cells in extracts of the immune cells. In other work, they have

been able to get skin cells to behave as if they were nerve cells.

"We can take a skin cell from your body and turn it directly into a cell type that you need to treat a particular disease," said Dr Philippe Collas, the leader of the team, whose work was published on May 1, 2002 in the respected journal *Nature Biotechnology*.
The technique being developed would allow skin cells from a patient to be turned directly into other types of cells without having to revert first to an embryonic state and without needing women's eggs.

The Norwegian researchers told the British wire news service *Reuters* that "the beauty of our system is that we are not working with embryos or dealing with stem cells at all".

In principle, it would be a one-day procedure. The patient would come in and give a skin biopsy to the laboratory to reprogramme and the day after, you could put the cells back into the patient. The technique would have immediate applications in cancer. Dr Collas and his group are also looking at making insulin-secreting pancreatic cells.

The approach will aid investigation of the mechanisms by which adult stem cells revert to cells capable of differentiating into other types of cells, with potential use in therapies for conditions like diabetes, Parkinson's disease and heart disease. From a clinical perspective, approaches based on this technology would allow replacement cells to be generated that are compatible with a patient's immune system, without the ethical problems of generating or destroying embryos.

Biotechnology in agriculture

It is in commercial crop farming that biotechnology has made the greatest impact to date. Some of these developments are:

Disease resistance

Many herbicides and insecticides are applied liberally during the growing of many high production crops because they are susceptible to fungal diseases and insect attacks. There are many plants in nature that have resistance to fungal disease and insect damage. When the gene that produces the resistance can be isolated, it can be transferred to other plants. So far, there has not been much success in transferring such resistance to food crops. However, there has been success with making soyabean resistant to the herbicide Roundup. A gene has been introduced into this patented soyabean, allowing the crop in the field to tolerate the herbicide Roundup when it is sprayed to kill the weeds.

Frost-free plants

Many marine organisms found in freezing waters produce a secretion that has been called "anti-freeze protein" that protects the organisms, including fish, from damage by frost in the extreme cold. Scientists have isolated this gene and inserted it into strawberries and potatoes to keep them stable during late spring frost. There is much controversy over these crops because of the introduction of a foreign, non-plant gene into them.

Long-lasting tomatoes

Genetically modified tomatoes with brand names have now flooded the market. In the case of the Flavr-Savr tomato, no new gene is introduced but a gene in it that makes ethylene for the tomato to ripen is deactivated. This allows the tomatoes to mature on the plant until they turn pink – when they are picked. These tomatoes can be ripened as needed by exposing them to ethylene gas. Since the Flavr-Savr tomato does not make ethylene, its shelf life is increased tremendously. As a result, one gets a much cheaper product with a taste that is said to be indistinguishable from natural tomatoes. Rats fed with this tomato died within two weeks, and there is no data to prove that the Flavr-Savr is safe for human consumption.

Increased milk production

There is a great deal of controversy in the feeding of milk-producing cows with the

growth hormone bovine somatotropin or BST. This is a widespread practice in the industrial countries, particularly the United States. The ingestion of BST by cows increases their metabolism and raises their milk production. Cows given extra BST often develop mastitis of the udder, which is treated with large doses of antibiotics that often end up in the milk.

The Human Genome Project

This is a research effort of the United States Government that was initiated in 1990 by the Department of Energy and the National Institutes of Health to analyse the DNA of human beings. The project, intended to be completed in 15 years, proposed to identify the chromosomal location of every human gene, to determine each gene's precise chemical structure in order to show its function in health and disease, and to determine the precise sequence of nucleotides of the entire set of genes (the genome).

Another project was to address the ethical, legal, and social implications of the information obtained. The information gathered will be the basic reference for research in human biology and will provide fundamental insights into the genetic basis of human disease. The new technologies developed in the course of the project will be applicable in numerous biomedical fields. In 2000, the government and the private corporation Celera Genomics jointly announced that the project had been virtually completed, five years ahead of schedule.

The hereditary instructions inscribed in DNA guide the development of the human being from fertilised egg cell to death. In this project, chromosome maps were developed in various laboratories worldwide through a coordinated effort guided by the NHI. The genetic markers for more than 4,000 diseases caused by single mutant genes have been mapped.

To get an idea of the magnitude of this project, imagine a stack of 25,000 books. If each book is two centimetres thick, the stack would measure 50 metres, the height of a 15-storey building.

Consider locating a particular word within one of the books in the stack. For a molecular biologist, this would be like trying to find one particular gene in the human genome. Up to this point molecular biologists have mapped only a tiny fraction of the genome. The 23 pairs of human chromosomes are estimated to contain

between 50,000 and 100,000 genes, although it appears that only about 5% are ever transcribed.

In February 2001, two rival teams of scientists presented their first interpretations of the human genome, the set of DNA-encoded instructions that specify a person.

The two teams reported in separately published articles that there are far fewer human genes than thought – probably a mere 30,000 or so – only a third more than those found in the roundworm.

One team, Celera Genomics, has compiled a parts list of the proteins needed to make a person. This team was led by Dr J. Craig Venter, president of Celera Genomics in Rockville, USA. Its report appeared in a 48-page article in *Science*, based in Washington.

The other team, a publicly funded consortium, has traced the history of how the "junk" regions of the genome accumulated and has found that small elements of the junk may play a useful role.

They also discovered that human genes have been derived directly from bacteria. This team comprised academic centres, mostly in the United States and Britain but with members in France, Germany, China and Japan. The consortium is financed largely by the National Institutes of Health and the Wellcome Trust of London. Its version of the human genome was described in a 62-page article in *Nature*, based in London. The principal author is Dr Eric Lander of the Whitehead Institute in Cambridge, Massachussetts, USA.

The two teams announced in June 2000 that they had assembled the human genome, but it has taken them until now to analyse their findings.

The interpretation of the genome – identifying the genes, their functions and controls, and how they relate to human physiology and disease – is expected in time to revolutionise medicine by clarifying the mechanism of many diseases and generating new tests and treatments.

Physically, the genome is minuscule – two copies of it are packed into the nucleus of every ordinary human cell, each one of which is about a fifth the size of the

smallest speck of dust the eye can see. But the genome is vast in terms of its informational content.

Composed of chemical symbols designated by the four-letter alphabet of As, Ts, Cs, and Gs, the human genome is some 3.2 billion letters in length. If printed in standard type, it would cover 75,490 pages of a broadsheet newspaper.

The enormous task of decoding the genomic message began in 1990 and is now substantially complete, although both teams' versions of the genome are riddled with gaps.

A new study of the genetic road map of human development, reported in August 2002, is starting to begin making sense of the genetic information. This genome-wide study by a team led by Dr David Altshuler of the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology in Cambridge, USA, has produced a sort of topographical map of the landscape of the human genome.

For the most part, people are "very similar" in terms of their genes, *Reuters Health* quoted Altshuler as saying in an interview. He estimates that the genome sequence is about 99.9% identical across the human race.

The small amount of variation that does exist accounts for the differences between people, the study suggests, such as hair color and height. These variations are due to "different spellings" of particular genes, referred to as *single nucleotide polymorphisms* or SNPs.

Conclusion

In early 1997, newspaper headlines around the world reported the cloning of a sheep by scientists in Scotland, Britain, who named it "Dolly". This was followed closely by reports of the cloning of two rhesus monkeys by scientists in Oregon, the United States.

These striking examples of the power of the techniques for manipulating the DNA sparked enormous amounts of discussion. Experiments on nucleic acids involve the

separation of extremely small quantities of the components of a mixture of different molecular sizes and to detect the presence of nucleic acids.

Transnational corporations claim that biotechnology has the potential to contribute to providing sufficient food for the growing world population by more environmentally-sensitive means. There is a wide range of interest groups involved with, and concerned about, the development and application of genetic technologies.

There is also considerable public unease about some of the possible applications, as demonstrated by the recent debates over genetically-modified maize and soybeans. Whether biotechnology will ultimately have benefits that are felt to outweigh the risks, including those risks that are unpredictable in nature, is still unclear to most people. There is also a dearth of data on analyses of GM crops, foods and food products. To this end, consumer information and education is essential in order to make biosafety risk-benefit analyses.

What is important at this stage of the development of the technology is that the debate be broadened beyond the rather narrow confines of research and commercial organisations and regulatory systems. The debate about risks, benefits and acceptability needs to involve a wide range of groups and individuals, using innovative discussion techniques and building new and credible institutions for handling complex decision-making.

This can only happen with the commitment of companies, researchers and regulators to move outside their traditional spheres of influence and accept a greater range of opinions in shaping their work.

The idea of genetic engineering in humans often upsets the people because they are fearful that scientists, transnational corporations which spend billions of US dollars in such research, and governments, will start "playing God". Genetic manipulations of food crops have also caused great concern and continue to raise passionate debate.

As the rate of technological advance often exceeds the interest or ability to cope of the public, including the scientists, the people are unable to fully understand the technology and its implications. When such disequilibria occurs, activist groups are usually effective in bringing technological evaluation back into synchrony with society's interest and understanding.

A chronology of developments in biotechnology

1750 BC	• The Sumerians brew beer.
500 BC	• The Chinese use mouldy soybean curds as an antibiotic to treat boils.
AD 100	• Powdered chrysanthemum is used as an insecticide in China.
1590	• Janssen invents the microscope.
1663	• Hooke first describes cells.
1675	• Leeuwenhoek discovers bacteria.
1797	• Jenner inoculates a child with a viral vaccine to protect him from smallpox.
1830	• Proteins are discovered.
1833	• The first enzymes are isolated.
1855	• The *Escherichia coli* (E. coli) bacterium is discovered. It later becomes a major research, development and production tool for biotechnology.
1863	• Mendel, in his study of peas, discovers that traits are transmitted from parents to progeny by discrete, independent units, later called genes. His observations laid the groundwork for the field of genetics.
1869	• Miescher discovers DNA in the sperm of the trout.
1877	• Koch develops a technique for staining and identifying bacteria.
1878	• Laval develops the first centrifuge.
1879	• Fleming discovers chromatin, the rod-like structures inside the cell nucleus that later came to be called chromosomes.
1900	• *Drosophila* (fruit flies) are used in the early studies of genes.
1902	• The term "immunology" first appears.
1906	• The term "genetics" is introduced.
1911	• The first cancer-causing virus is discovered by Rous.
1914	• Bacteria are used to treat sewage for the first time in Manchester, England.
1915	• Phages, or bacterial viruses, are discovered.
1919	• The word "biotechnology" is used for the first time, by a Hungarian agricultural engineer.
1920	• Evans and Long discover the human growth hormone.

1928
- Fleming discovers penicillin, the first antibiotic.

1938
- The term "molecular biology" is coined.

1940
- American Oswald Avery demonstrates that the DNA is the "transforming factor" and is the material of genes.

1941
- The term "genetic engineering" is first used by Danish microbiologist A. Jost in a lecture on sexual reproduction in yeast at the technical Institute in Lwow, Poland.

1942
- The electron microscope is used to identify and characterise a bacteriophage – a virus that infects bacteria.

1944
- Waksman isolates streptomycin, an effective antibiotic for TB.

1946
- It is discovered that genetic material from different viruses can be combined to form a new type of virus, an example of genetic recombination.

1947
- McClintock discovers transposable elements, or "jumping genes," in corn.

1949
- Pauling shows that sickle cell anemia is a "molecular disease", resulting from a mutation in the protein molecule haemoglobin.

1950
- Artificial insemination of livestock using frozen semen (a long-time dream of farmers) is successfully accomplished.

1953
- *Nature* publishes a manuscript by James Watson and Francis Crick, describing the double helical structure of DNA, which marks the beginning of the modern era of genetics.

1954
- Cell-culturing techniques are developed.

1955
- An enzyme involved in the synthesis of a nucleic acid is isolated for the first time.

1956
- The fermentation process is perfected in Japan.
- Kornberg discovers the enzyme DNA polymerase I, leading to an understanding of how DNA is replicated.

1958
- Sickle cell anemia is shown to occur due to a change of a single amino acid.

1959
- Systemic fungicides are developed. The steps in protein biosynthesis are delineated.

Also in the 1950s
- Discovery of interferons, the first synthetic antibiotic.

1960
- Exploiting base pairing, hybrid DNA-RNA molecules are created. Messenger RNA is discovered.

1964
- The International Rice Research Institute in the Philippines starts the Green Revolution with new strains of rice that double the yield of previous strains, if given sufficient fertilisers.

1965
- Harris and Watkins successfully fuse mouse and human cells.

1966
- The genetic code is cracked, demonstrating that a sequence of three nucleotide bases (a condon) determines each of 20 amino acids.

1967
- The first automatic protein sequencer is perfected.

1969
- An enzyme is synthesised in vitro for the first time.

1970
- Specific restriction nucleases are identified, opening the way for gene cloning.
- First complete synthesis of a gene.

1971

Discovery of restriction enzymes that can be used to cut and splice genetic material.

1972
- The DNA composition of humans is discovered to be 99% similar to that of chimpanzees and gorillas.
- Initial work with embryo transfer.

1973
- Stanley Cohen and Herbert Boyer perfect genetic engineering techniques to cut and paste DNA (using restriction enzymes and ligases) and reproduce the new DNA in bacteria.

1974
- The National Institutes of Health (NIH) forms a Recombinant DNA Advisory Committee to oversee recombinant genetic research.

1975
- Asilomar Conference (moratorium on genetic engineering research).
- The first monoclonal antibodies are produced.

1976
- The tools of recombinant DNA are first applied to a human inherited disorder.
- Molecular hybridisation is used for the prenatal diagnosis of alpha thalassemia. Yeast genes are expressed in E. coli bacteria.
- DNA sequencing discovered; first working synthetic gene.

1977
- First expression of the human gene in bacteria.
- Methods for reading DNA sequence using electrophoresis are discovered.

1978
- High-level structure of virus first identified.
- Recombinant human insulin is first produced.

- North Carolina scientists show it is possible to introduce specific mutations at specific sites in a DNA molecule.

1979

Human growth hormone is first synthesised.

Also in the 1970s

- First commercial company founded to develop genetically engineered products.
- Discovery of polymerases.
- Techniques for rapid sequencing of nucleotides are perfected.
- Gene targeting and RNA splicing are carried out.

1980

- The US Supreme Court, in the landmark case *Diamond vs Chakrabarty*, approves the principle of patenting genetically engineered life forms, which allows the Exxon oil company to patent an oil-eating microorganism.
- The US patent for gene cloning is awarded to Cohen and Boyer.
- The first gene synthesising machines are developed. Researchers successfully introduce a human gene – one that codes for the protein interferon – into a bacterium.

1981

- Scientists at Ohio University produce the first transgenic animals by transferring genes from other animals into mice.
- Chinese scientists become the first to clone a fish - a golden carp.
- Applied Biosystems Inc introduces the first commercial gas phase protein sequencer, dramatically reducing the amount of protein samples needed for sequencing.

1983

- The Polymerase Chain Reaction (PCR) technique is conceived. PCR, which uses heat and enzymes to make unlimited copies of genes and gene fragments, later becomes a major tool in biotech research and product development worldwide.
- The first genetic transformation of plant cells by TI plasmids is performed.
- The first artificial chromosome is synthesised.
- The first genetic markers for specific inherited diseases are found.

1984

- The DNA fingerprinting technique is developed. The first genetically engineered vaccine is developed.
- The entire genome of the HIV virus is cloned and sequenced.

1985

- Genetic marking found for kidney disease and cystic fibrosis.
- Genetic fingerprinting enters the courtroom.

- Genetically engineered plants resistant to insects, viruses and bacteria are field tested for the first time.
- The NIH approves guidelines for performing experiments in gene therapy on humans.

1986

- A University of California, Berkeley, chemist describes how to combine antibodies and enzymes (abzymes) to create pharmaceuticals.
- The first field tests of genetically engineered plants (tobacco) are conducted.
- The Environmental Protection Agency approves the release of the first genetically engineered crop - gene-altered tobacco plants.
- Kidney transplant rejection.
- Recombinate rAHF, a blood-clotting Factor VIII for the treatment of hemophilia A, is approved.

1987

- First field trials of a genetically altered bacterium. Frostban, a genetically altered bacterium that inhibits frost formation on crop plants, is field tested on strawberry and potato plants in California, the first authorised outdoor tests of an engineered bacterium.

1988

- Harvard molecular geneticists are awarded the first US patent for a genetically altered animal – a transgenic mouse.
- A patent for a process to make bleach-resistant protease enzymes to use in detergents is awarded.
- Congress approves funds the Human Genome Project, a massive effort to map and sequence the human genetic code as well as the genomes of other species.

1989

- First field trial of a recombinant viral crop protectant.
- Epogen is approved for the treatment of the renal disease anaemia.
- The gene responsible for cystic fibrosis is discovered.
- Alferon N is approved for treatment of genital warts.
- Kogenate, which replaces blood-clotting factor VIII for the treatment of hemophilia A, is approved.

Also in the 1980s

- Studies of DNA used to determine evolutionary history.
- Recombinant DNA animal vaccine approved for use in Europe.
- Use of microbes in oil spill cleanup – bioremediation technology is put to use.
- Ribozymes and retinoblastomas are identified.

1990

- Chy-Max, an artificially produced form of chymosin, an enzyme for cheese-making, is introduced. It is the first product of recombinant DNA technology in the US food supply.
- The Human Genome Project – an international effort to map all of the genes in the human body – is launched.
- The first federally approved gene therapy treatment is performed successfully in the US on a four-year-old girl suffering from an immune disorder.
- The first successful field trial of genetically engineered cotton plants is conducted. The plants had been engineered to withstand the use of the herbicide Bromoxynil.
- The first transgenic dairy cow – used to produce human milk proteins for infant formula – is created.

1991

- Neupogen is approved for the treatment of low white blood cells in chemotherapy patients.
- Leukine, used to replenish white blood cell counts after bone marrow transplants, is approved.
- Eredase is approved for the treatment of Type 1 Gaucher's disease.

1992

- Proleukin® is approved for the treatment of renal cell cancer.
- American and British scientists unveil a technique for testing embryos *in vitro* for genetic abnormalities such as cystic fibrosis and hemophilia.

1993

- Betaseron is approved as the first treatment for multiple sclerosis in 20 years.
- The FDA declares that genetically engineered foods are "not inherently dangerous" and do not require special regulation.

1994

- The Flavr-Savr tomato – the first genetically engineered whole food approved by the US Food and Drug Authority, is on the market.
- The first breast cancer gene is discovered.
- Approval is given to a genetically-engineered version of human DNAase, which breaks down protein accumulation in the lungs of CF patients.

1995

- The first baboon-to-human bone marrow transplant is performed on an AIDS patient.
- The first full gene sequence of a living organism other than a virus is completed for the bacterium *Hemophilus influenzae*.
- Gene therapy, immune system modulation and genetically engineered

antibodies enter the clinics in the war against cancer.

1996

- The discovery of a gene associated with Parkinson's disease provides an important new avenue of research into the cause and potential treatment of the debilitating neurological ailment.

1997

- Scottish scientists report cloning a sheep, named Dolly, using DNA from adult sheep cells.
- A group of Oregon researchers claims to have cloned two Rhesus monkeys.
- A new DNA technique combines PCR, DNA chips and a computer program providing a new tool in the search for disease-causing genes.
- Abbott HTLV-1/HTLV-II EIA is approved for the detection of HTLV-I/HTLV-II antibodies in serum or plasma.

1998

- University of Hawaii scientists clone three generations of mice from nuclei of adult ovarian cumulus cells.
- Embryonic stem cells can be used to regenerate tissue and create disorders mimicking diseases.
- Scientists at Japan's Kinki University clone eight identical calves using cells taken from a single adult cow
- The first complete animal genome for the elegans worm is sequenced.
- A rough draft of the human genome map is produced, showing the locations of more than 30,000 genes.

Also in the 1990s

- The first conviction of a person, through the use of genetic fingerprinting, is obtained in Britain.
- A gene that clearly participates in the normal process of regulating weight is isolated.
- It is discovered that hereditary colon cancer is caused by a defective DNA repair gene.
- Genetically engineered rabies vaccine tested in raccoons.
- Genetically engineered biopesticide is approved for sale in the United States.
- Patents issued for mice with specific transplanted genes.
- First European patent issued for genetically-engineered mouse sensitive to carcinogens.
- Breast cancer susceptibility genes cloned.

About **ERA Consumer**

The Education and Research Association for Consumers, Malaysia (ERA Consumer, Malaysia) is a voluntary, non-profit and non-political organisation that was founded in Ipoh, Perak in 1985. ERA Consumer is a registered membership organisation under the Malaysian Societies Act of 1966. It was set-up to undertake and promote the task of developing critical consciousness on public-related issues out of the larger socio-economic issues.

ERA Consumer is a dynamic institution that is constantly responding to and developing its services according to the needs and demands of the people. It aims to create awareness among the public on issues that are effecting their lives, through research and educational programmes by undertaking independent, authoritative, balanced research on public issues; carrying out public education projects; making policy recommendations to the government & international institutions; building solidarity and understanding among NGOs in Malaysia and society at large, and to increase South-South relations and North-South understanding. ERA Consumer's components and main programmes are consumer issues; human rights education; food, trade and economics.

Published by:

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ISBN : 983-2518-18-0

Edition June 2003

Printed by : Syarikat Asas Jaya

ISBN 983-2518-18-0
9 789832 518181