CHAPTER

23 BIOTECHNOLOGY

Animation 23 : Biotechnology
Source & Credit: Wikispaces

Since Mendel's work was rediscovered in 1900, geneticists have made startling advances which have led to a new era of DNA technology. Modem techniques enable desired substance, for example insulin. Not very long ago, people with insulin dependent they receive human insulin, a product of biotechnology. Since the 1980s, biotechnology has produced drugs and vaccines to curb human illnesses.

Genetically, engineered bacteria have been used to clean up environmental pollutants, increase the fertility of the soil, and kill insect pests. Biotechnology also extends beyond multicellular organisms. It is now possible to alter the genotype and subsequently the phenotype of plants and animals. Indeed, gene therapy in humans, attempting to repair a faulty gene is already undergoing clinical trials. There are those who are opposed to manipulation of genes for any reason. Although, there have been no ill effects as yet, they fear the possibility of health and ecological repercussions in the future.

Cloning of a gene

Produces many identical copies. Recombinant DNA technology is used when a very large quantity of a gene is required. The use of polymerase chain reaction (PCR) creates a lesser number of copies within a laboratory test tube.

Recombinant DNA Technology

Recombinant DNA technology popularly known as genetic engineering aims at synthesizing recombinant DNA which contains DNA from two different sources. In order to produce recombinant DNA, the following are required:

1. Gene of interest, which is to be cloned.
2. Molecular scissors to cut out the gene of interest.
3. Molecular carrier or vector, on which gene of interest could be placed.
4. The gene of interest alongwith the vector is then introduced into an expression system, as a result of which a specific product is made.

How to get a gene?

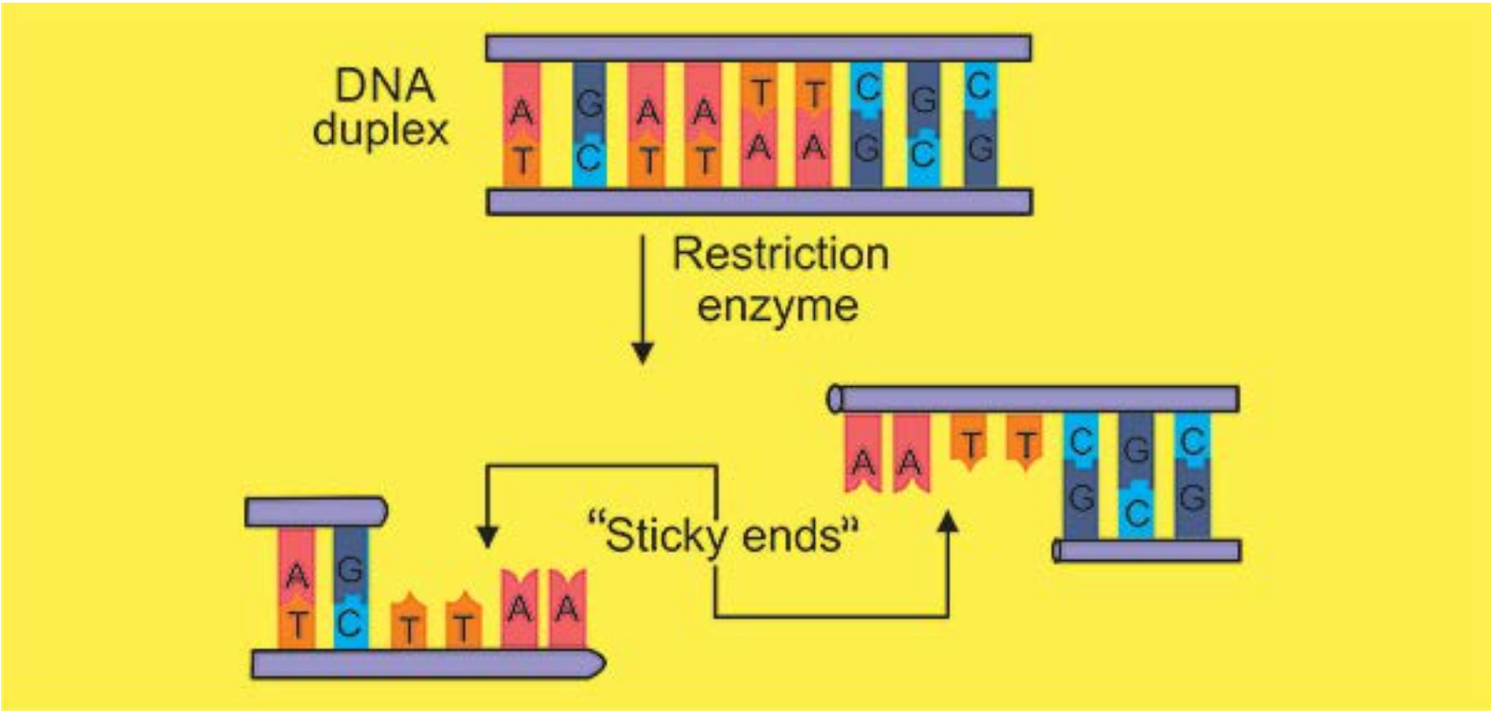
There are three possible ways to get the gene of interest.
(a) to isolate it from the chromosome
(b) to synthesize it chemically, and
(c) to make it from mRNA

Genes can be isolated from the chromosomes by cutting the chromosomes on the flanking sites of the gene using special enzymes known as restriction endonucleases. If, however, the genes are small, they can also be synthesized in the laboratory. Another very common method of getting the gene is to synthesize it in the laboratory from messenger RNA, using reverse transcriptase. This DNA molecule is called complementary DNA (cDNA).

Molecular Scissors: Restriction Endonucleases

These are natural enzymes of bacteria, which they use for their own protection against viruses. The restriction enzyme cuts down the viral DNA, but does no harm to the bacterial chromosofhe. They are called restriction enzymes because they restrict the growth of viruses. In 1970, Hamilton O. Smith, at Johns Hopkins University, isolated the first restriction enzyme. Bacteria produce a variety of such restriction enzymes, which cut the DNA at very specific sites characterized by specific sequence of four or six nucleotides arranged symmetrically in the reverse order. Such sequences are known as palindromic sequences. So far more than 400 such enzymes have been isolated out of which about 20 are frequently used in recombinant DNA technology.

EcoRl, a commonly used restriction enzyme, cuts double-stranded DNA when it has this sequence of bases at the cleavage site (Fig. 23.1). Notice there is now a gap into which a piece of foreign DNA can be placed, if it ends in bases complementary to those exposed by the restriction enzyme. The single stranded but complementary ends of the two DNA molecules are called “sticky ends” because they can bind by complementary base pairing. They, therefore, facilitate the insertion of foreign DNA into vector DNA.



Fig, 23.1 Restriction enzyme ECoRI, cuts this specific sequence of nucleotides in such a way that sticky ends are produced.

Molecular Carrier: Vector

To make recombinant DNA, one often begins by selecting a vector, the means by which recombinant DNA is introduced into a host cell. One common type of vector is a plasmid. Plasmids were discovered by investigators studying the sex life of the intestinal bacterium *Escherichia coli*.

Plasmids are natural extra-chromosomal circular DNA molecules which carry genes for antibiotic resistance and fertility etc. One of the plasmids discovered earlier pSC 101 has antibiotic resistance gene for tetracycline, whereas pBR 322 has antibiotic resistance genes for tetracycline as well as ampicillin. Inserting gene of interest in tetracycline resistant gene of plasmid pBR 322 would enable separating out colonies of bacteria in a medium containing ampicillin and vice versa.

Recombinant DNA

For preparation of a recombinant DNA, the plasmid is cut with the same enzyme which was used for isolation of the gene of interest (Fig. 23.2). The gene of interest (insulin) is then joined with the sticky ends produced after cutting the plasmid with the help of another special enzyme knqwn as DNA ligase. This enzyme seals the foreign piece of DNA into the vector. Now the two different pieces of DNA have been joined together, which is now known as recombinant DNA or chimaeric DNA

Animation 23.1: Recombinant DNA
Source & Credit:Pinterest

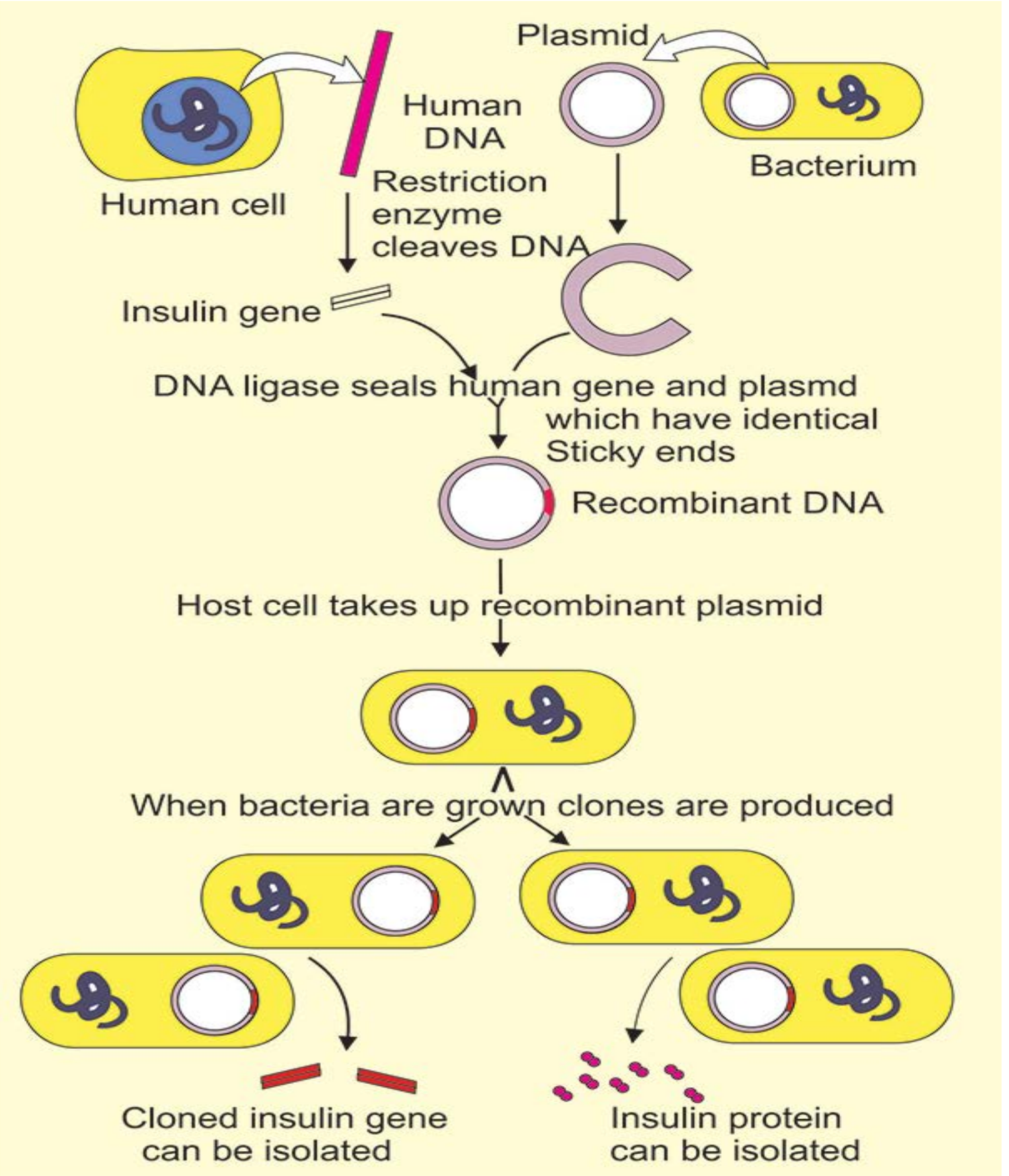


Fig. 23.2 Cloning of a gene.

Expression of the Recombinant DNA

A clone can be a large number of molecules (i.e. cloned genes) or cells (i.e. cloned bacteria) or organisms that are identical to an original specimen. Fig. 23.3 compares the use of a plasmid and a virus to clone a gene. Bacterial cells take up recombinant plasmid, especially, if they are treated with calcium chloride to make them more permeable. Thereafter, as the cell reproduces, a bacterial clone forms and each new cell contains at least one plasmid. Therefore, each of the bacteria contains the gene of interest, which will express itself and make a product. From this bacterial clone, the cloned gene can be isolated for further analysis, or protein product can be separated (Fig 23.2). Besides plasmids, the DNA of bacterial viruses (for example, lambda phage) can also be used as a vector. After lambda phage attaches to a host bacterium, recombinant DNA is released from the virus and enters the bacterium. Here, it will direct the reproduction of many more viruses. Each virus in bacteriophage clone contains a copy of the gene being cloned (Fig. 23.3).

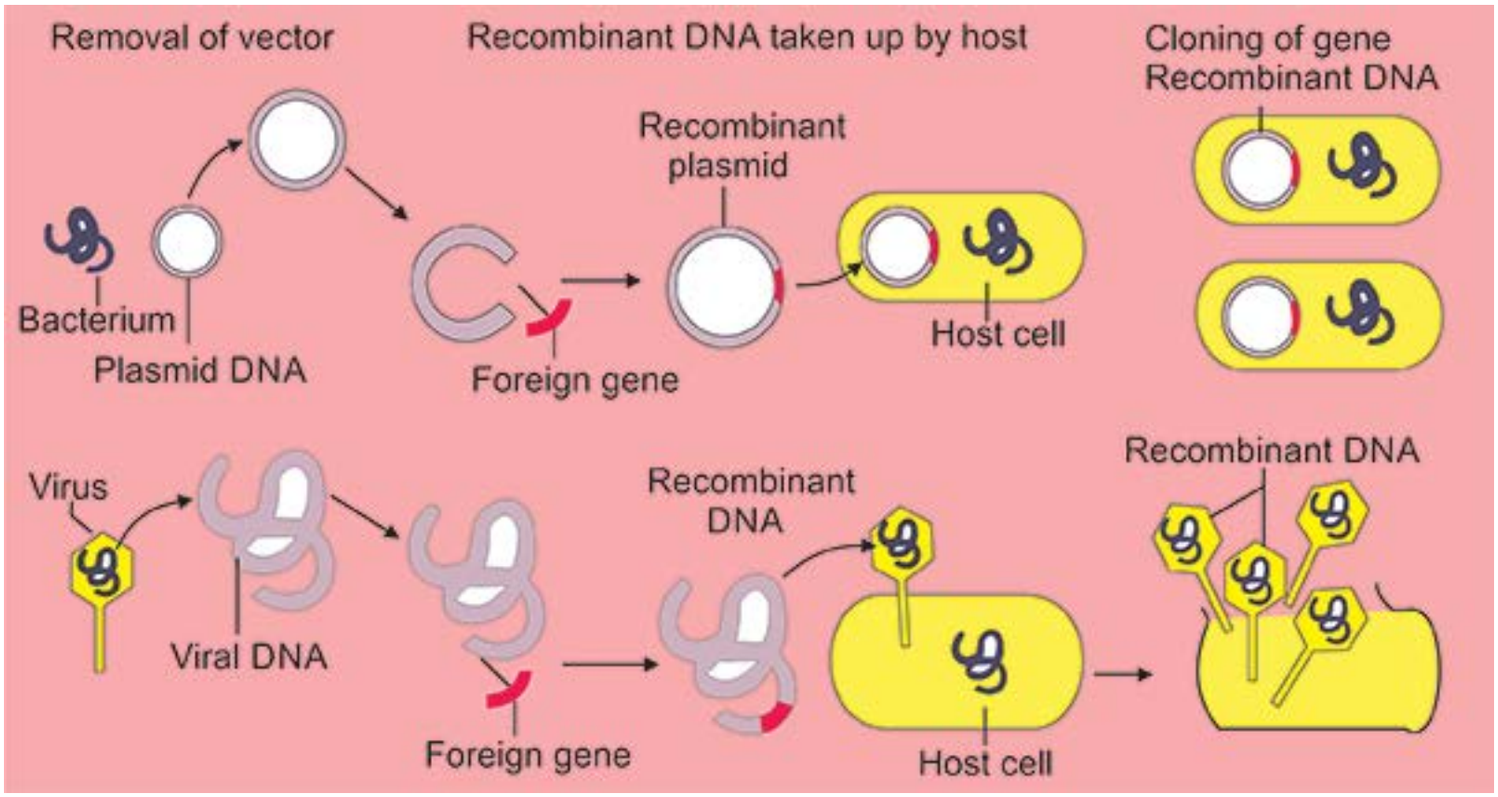


Fig. 23.3 Plasmid DNA (upper part of figure) as well as viral DNA (lower part of the figure) can be used as vectors for cloning gene of interes

Genoiftic Library

A genome is a full set of genes of an individual. A genomic library is a collection of bacterial or bacteriophage clones, each clone containing a particular segment of DNA from the source cell. For making a genomic library, an organism's DNA is simply sliced up into pieces, and pieces are put into vectors (i.e. plasmids or viruses) that are taken up by host bacteria as shown in Fig. 23.3. The entire collection of bacterial or bacteriophage clones that result contains all the genes of that organism.

A particular probe can be used to search a genetic library for a certain gene. A probe is a single stranded nucleotide sequence that will hybridize (pair) with a certain piece of DNA. Location of the probe is possible because the probe is either radioactive or fluorescent. Bacterial cells, each carrying a particular DNA fragment, can be plated onto agar in a petri dish. After the probe hybridizes into the gene of interest, the genes can be isolated from the fragment (Fig 23.4). Now this particular fragment can be cloned further or even analyzed for its particular DNA sequence.

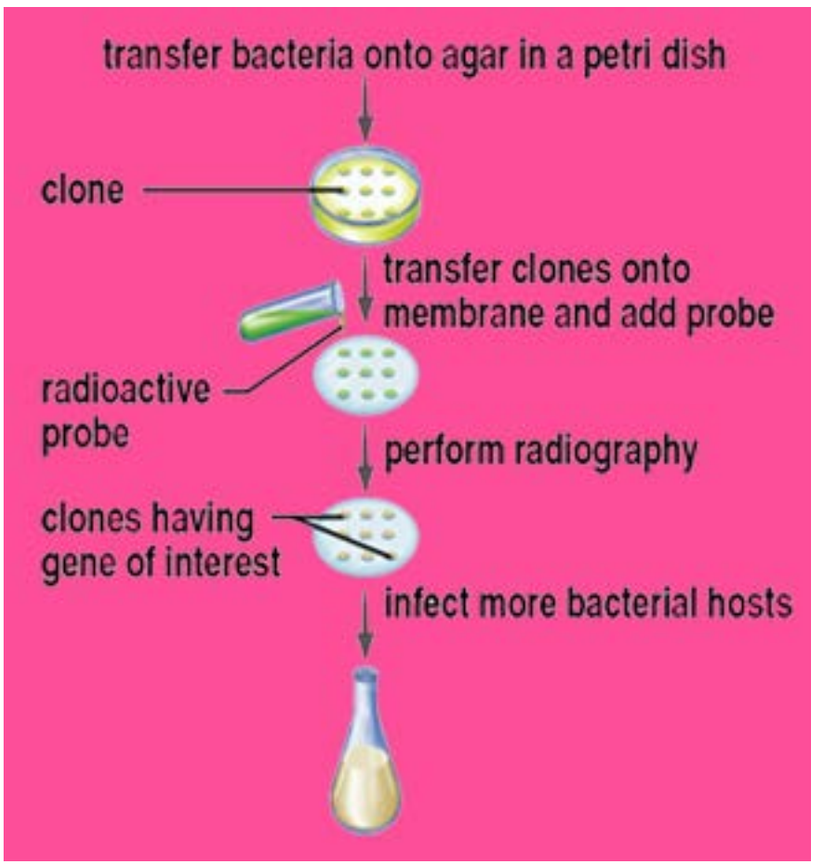


Fig 23.4 Identification of a cloned gene

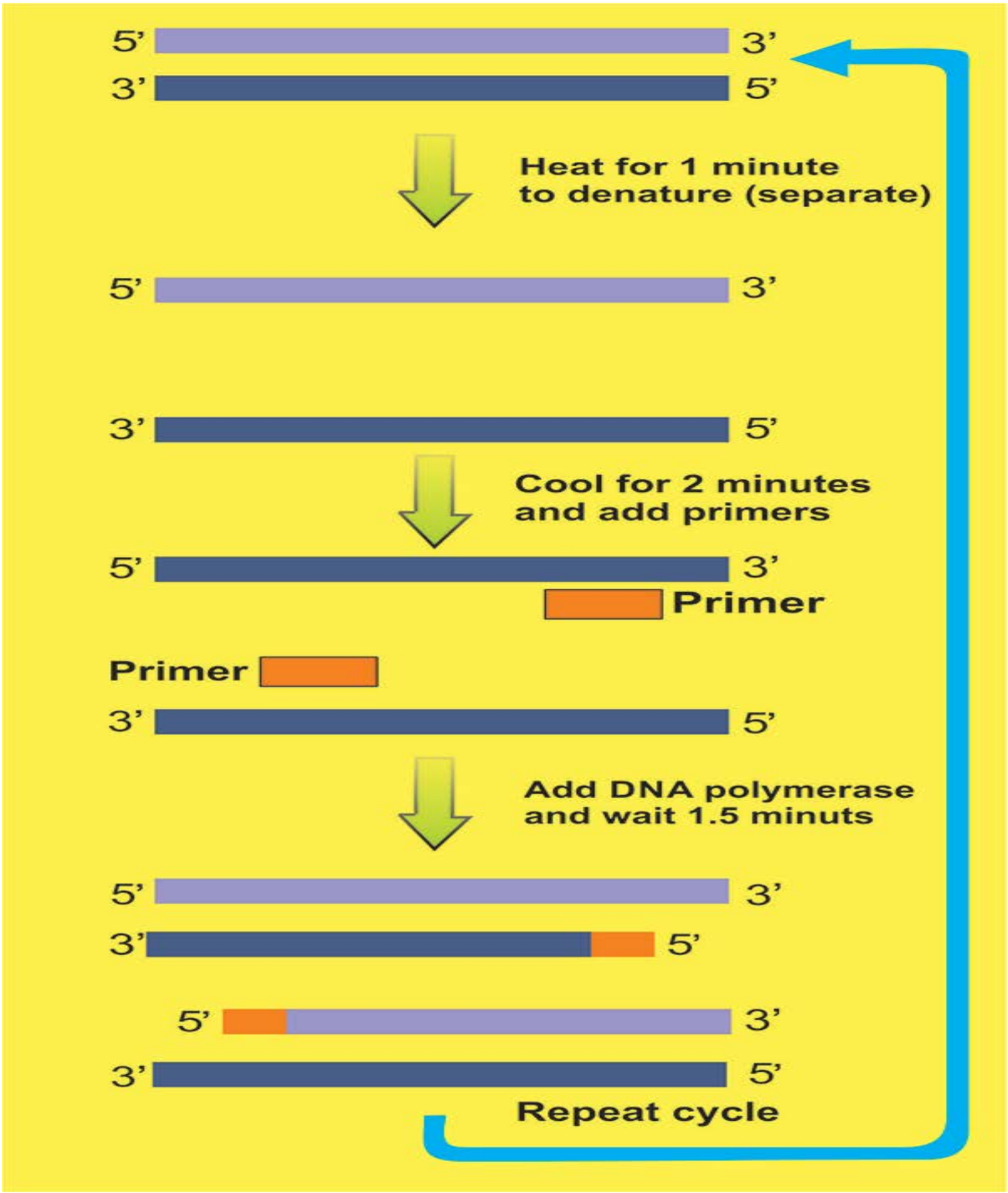


Fig 23.5 Polymerase chain reaction (PCR)

The polymerase Chain Reaction

Kary B. Mullis developed the polymerase chain reaction (PCR) in 1983. Earlier methods of obtaining multiple copies of a specific sequence of DNA were time consuming and expensive. In contrast, PCR can create millions of copies of a single gene or any specific piece of DNA quickly in a test tube. PCR is very specific - the targeted DNA sequence can be less than one part in a million of the total DNA sample. .This means that a single gene or smaller piece of DNA, among all the human genes can be amplified (copied) using PCR.

PCR takes its name from DNA polymerase, the enzyme that carries out DNA replication in a cell. It is considered a chain reaction because DNA polymerase will carry out replication over and over again, until there are millions of copies of the desired DNA. PCR does not replace gene cloning, which is still used whenever a large quantity of gene or protein product is needed.

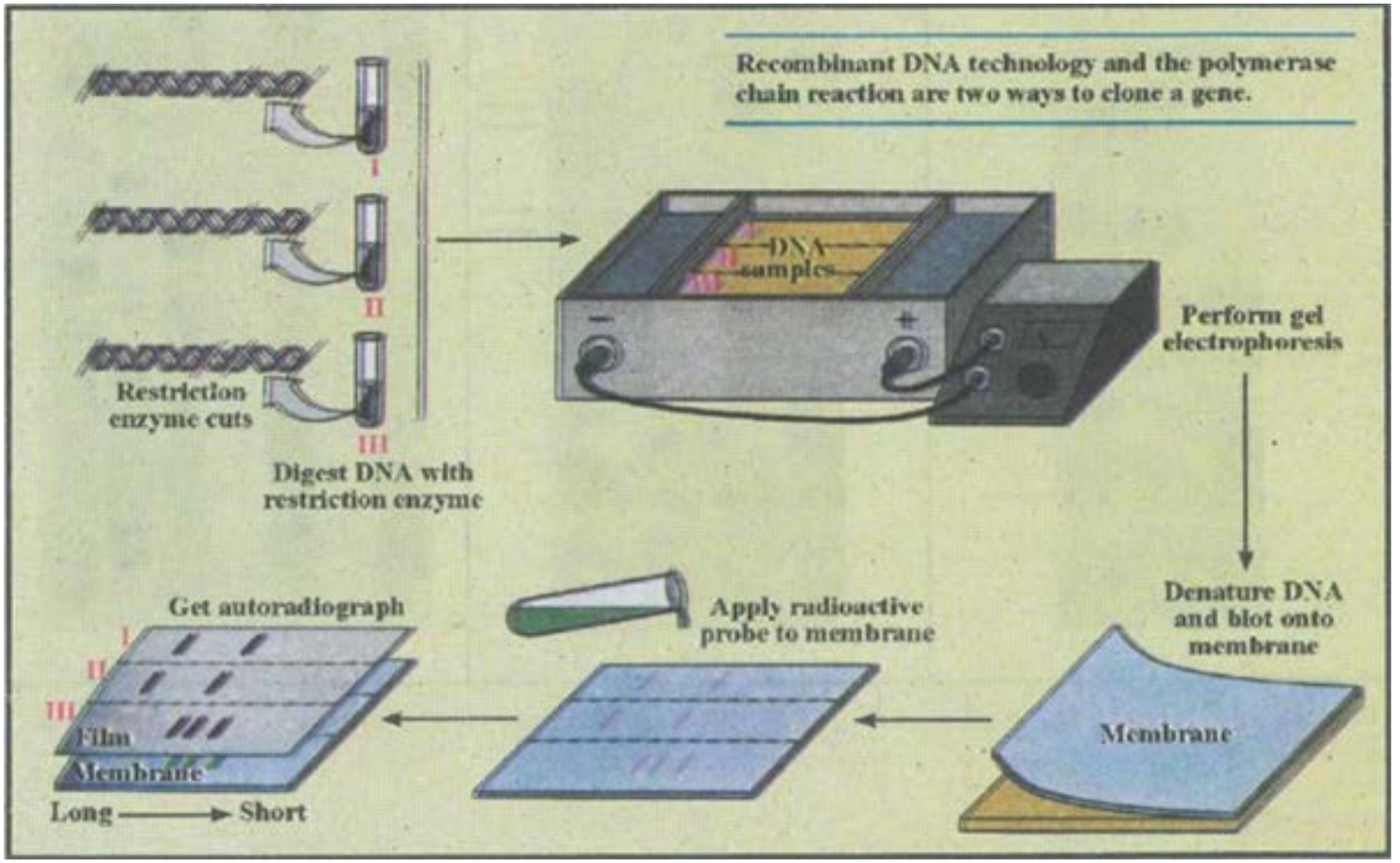
Before carrying out PCR, primers - sequences of about 20 bases that are complementary to the bases on either side of the "target DNA" - must be available. The primers are needed because DNA polymerase does not start the replication process; it only continues or extends the process. After the primers bind by complementary base pairing to the DNA strand, DNA polymerase copies the target DNA (Fig 23.5) .

DNA polymerase used is temperature - insensitive (thermostable) enzyme extracted from the bacterium *Thermus aquaticus*, which lives in hot springs. Commonly, this enzyme is also known as **Taq polymerase**. It can withstand high temperature, which is used to separate double stranded DNA, therefore, replication need not be interrupted by the need to add more enzyme. PCR is done these days in an automatic PCR machine or thermocycler, which is a routine piece of equipment in any laboratory.

Animation 23.2 : PCR
Source & Credit: members.jcom

Analyzing DNA

The entire genome of an individual can be subjected to DNA finger printing, a process described in Fig. 23.6. The genome is treated with restriction enzymes, which results in a unique collection of different sized fragments. Therefore, restriction fragment length polymorphism (RFLPs) exists between individuals. During a process called gel electrophoresis, the fragments can be separated according to their lengths (molecular weight or size), and the result is a number of bands that are so close together that they appear as a smear. However, the use of probes for genetic markers produces a distinctive pattern that can be recorded on X-ray film.



Fif 23.6 DNA fingerprinting. Three samples of DNA(I , II , IIi)were cut with a restriction enzyme and run on agarose gel. The gel pattren was then transferred to a membrane and DNA was denatured. The denatured DNA on the paper was hybridized with radioactive probe. Since the radioactive probes and complemetary arrangement of bases to the original DNA ,all DNA fragments were labelled , which appeared as black bands with autogradiagram.

The DNA from a single sperm enough to identify a suspected rapist. Since DNA is inherited, its finger print resembles that of one's parents. DNA finger printing successfully identified the remains of a teenager who had been murdered eight years before because the skeletal DNA was similar to that of the parent's DNA.

In Fig. 23.7 are given some DNA finger prints. The figure 23.7 (a) shows comparison of child's finger print with that of his parents. The child has received DNA from both of his parents. Arrows indicate that some bands in him are like his father, some like his mother. Some bands are. however, unique to him, which do not match with any of the parents.

Fig. 23.7 (b) shows a case of disputed parenthood. Two persons F_1 and F_2 claim to be the father of child C, whose mother's finger print is given under M. The child has received DNA from both of his parents. Obviously $F_1$is not the real father.

The arrows on left side show common bands between mother and child while those on right show common bands between the father and the child.

Fig. 23.7(c) shows DNA finger prints which have been presented as forensic evidence. A criminal on a deserted place assaulted a woman. She scratched his face in her defence but he murdered her and ran away. Forensic scientist recovered murder's hair and skin cells from underneath her nails. They prepared DNA finger prints from blood of victim, from murderer's skin and hair, and from three suspects blood. Can you compare them for specific DNA sequence and tell who is in guilty and who is not? The suspect 1 has finger prints, which is similar to linger print from skin cells taken from underneath nails of the victim.Therefore suspect 1 is the culprit. The suspect 2 and 3 are not.

PCR amplification and analysis can be used (1) to diagnose viral infections, genetic disorders, and cancer (2) in forensic laboratories to identify criminals; and (3) to determine the evolutionary history' of human population. It has been possible to sequence DNA taken from a 76,000 years old mummified human braiti and from a 17 to 20 million years old plant fossil following PCR amplification.

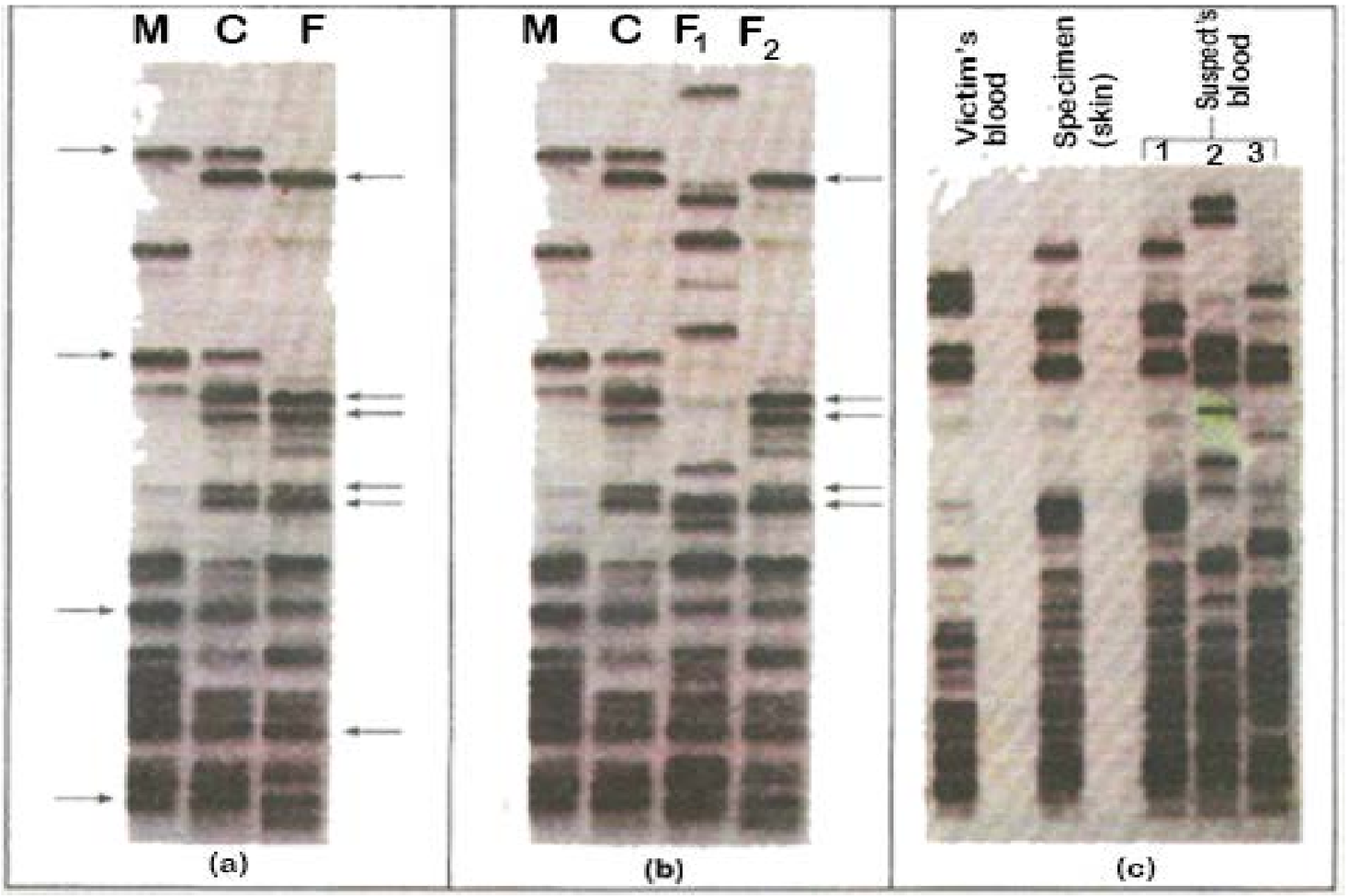


Fig 23.7(a) Comparison of a child's DNA fingerprint (c) with his parent's DNA fringerpints (Mand F), *(b) DNA fingerprints as evidence for paternity.* *(c) DNA Test - a powerful tool of forensic science.*

Gene Sequencing

In the late 1970s, methods were developed that allowed the nucleotide sequence of any purified DNA fragement to be determined simply and quickly. The main principle of these methods is :

1. To generate pieces of DNA of different sizes all starting from the same point and ending at different points.
2. Separation of these different pieces of DNA on agarose gel.
3. Reading of sequence from the gel.

For generation of different sized DNA fragments, two methods arc generally used. One is Sanger's,method in which dideoxyribonucleoside triphosphates arc used to terminate DNA synthesis at different sites. The other method is known as Maxam-Giibcrt method in which DNA threads are chemically cut into pieces of different sizes

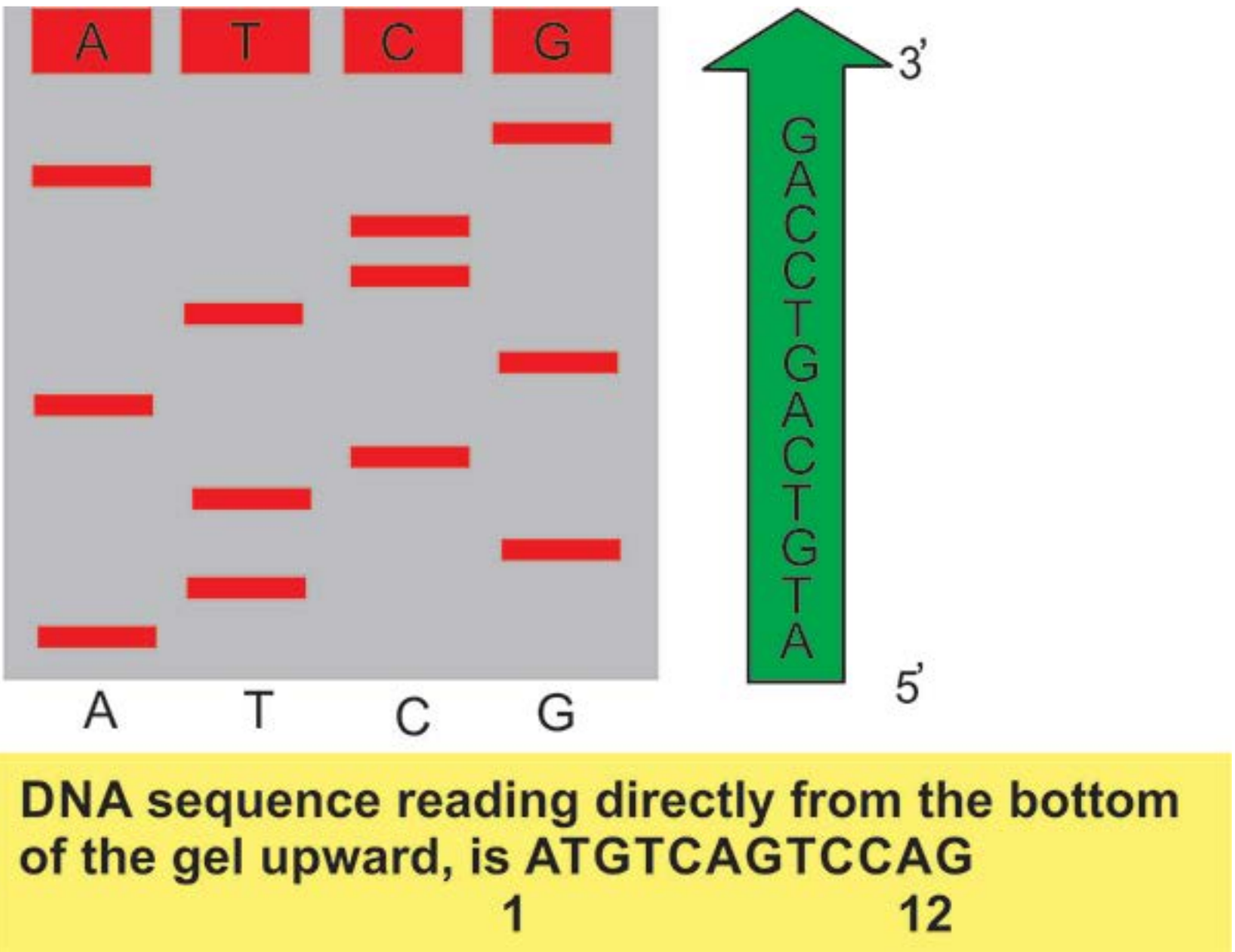


Fig 23.8 The enzymatic or dideoxy method of sequencing DNA

Fig 23.8 shows typical gel obtained after dideoxy method. The volume of DNA sequence information is now so large that powerful computers must be used to store and analyze it. DNA sequence is now completely automated, robotic devices mix the reagents and then load, run and read the order of the nucleotide bases from the gel. This is facilitated by using chain terminating nucleotides that are each labelled with a different colored fluorescent dye; in this case, all four synthesis reactions can be performed in the same tube, and the products can be separated in a single lane of a gel. A detector positioned near the bottom of the gel reads and records the color of fluorescent label on each band as it passes through a laser beam. A computer then reads and stores this nucleotide sequence.

Owing to the automation of DNA sequencing, the genomes of many organisms have been sequenced. These include plant chloroplasts and animal mitochondria, large number of bacteria, many of the yeasts, a nematode worm. *Drosophila*, the model plant *Arabidopsis*, the mouse and human. Researchers have also deduced the complete DNA sequence of a variety of human pathogens.

THE HUMAN GENOME PROJECT

The human genome project is massive effort originally founded by the U.S. government and now increasingly by U.S. pharmaceutical companies to map the human chromosomes. Many non-profit and for profit biochemical laboratories around the world are now involved in the project which has two primary goals.

Animation 23.3: Human Genome Project
Source & Credit: CRDD

The first goal is to construct a genetic map of the human genome. The aim is to show the sequence of genes along the length of each type of chromosome, such as depicted for the X chromosome in Fig 23.9. When the DNA sequence of human chromosome no. 22, one of the smallest human chromosomes, was completed in 19.99, it became possible for the first time to see exactly how genes are arranged along an entire vertebrate chromosome. With the publication of the entire human genome in 2001, the genetic landscape of all human chromosomes suddenly came into sharp focus. The sheer quantity of information provided by the human genome project is unprecedented in biology. The human genome is 25 times larger than any other genome sequenced so far.

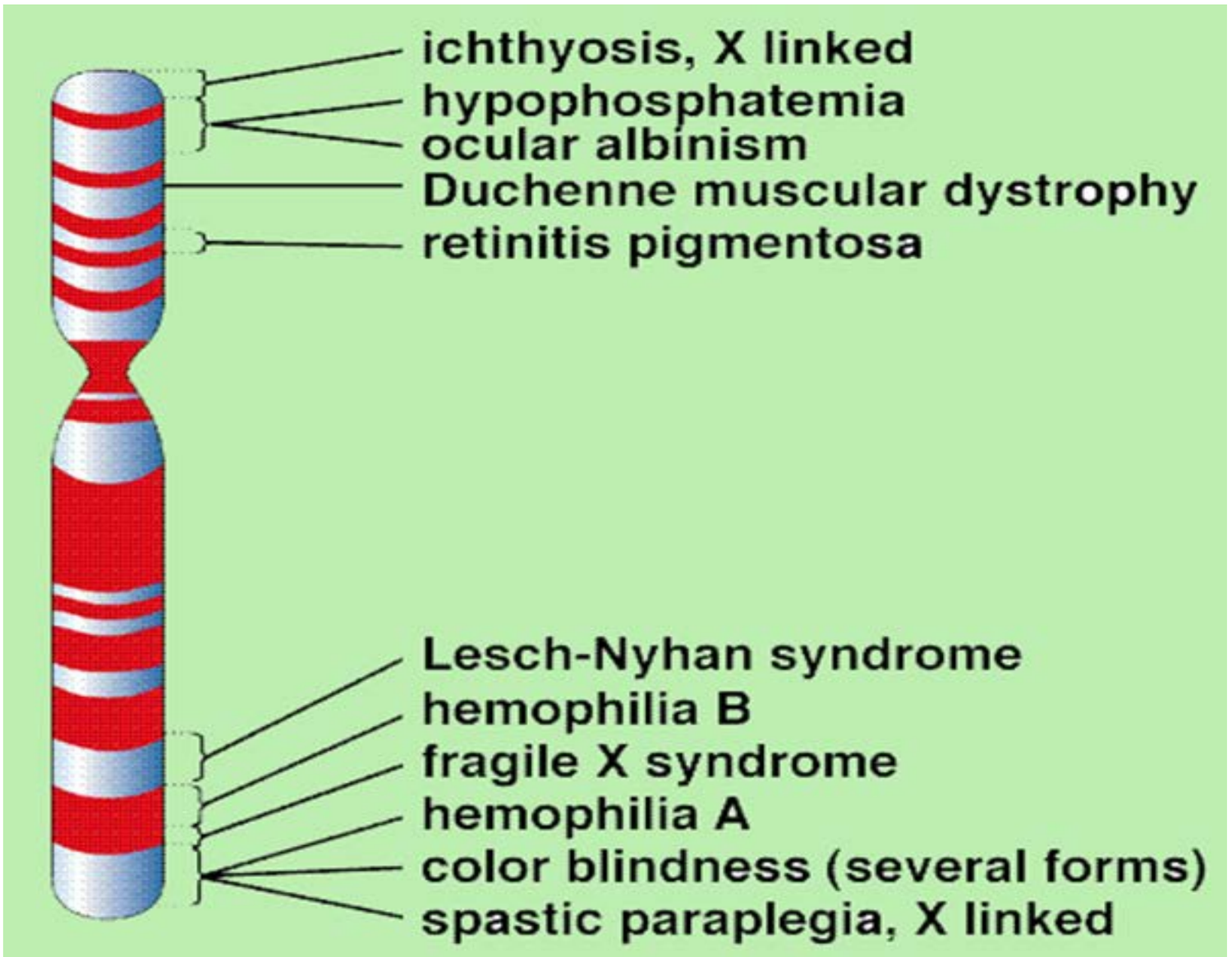


Fig 23.9 Genetic map of X chromosome

The map for each chromosome is presently incomplete, and in many instances scientists rely on the placement of RFLPs. These sites eventually allow scientist to pinpoint disease causing genes because a particular RFLP and a defective gene are often inherited together. For example it is known that persons with Huntington disease have a unique site where a restriction enzyme cuts DNA. The test for Huntington disease relies on this difference from the normal.

The second goal is to construct a base sequence map. There are three billion base pairs in the human genome and it is estimated it cohld take an encyclopaedia of 200 volumes, each with 1000 pages, to list all of these. Yet this goal has been reached and all the chromosomes have been sequenced.

BIOTECHNOLOGY PRODUCTS

Today bacteria, plants and animals are genetically engineered to produce biotechnology products. Organisms that have a foreign gene inserted into them are called **transgenic organisms.**

Transgenic Bacteria

Recombinant DNA technology is used to produce bacteria that reproduce in large vats called **bioreactors**. If the foreign gene is replicated and actively expressed, a large amount of protein product can be obtained. Biotechnology products produced by bacteria, such as insulin, human growth hormone, tissue plasminogen activator, haemophilia factor Vm, and hepatitis B vaccine are now in the market.

Transgenic bacteria have been produced to promote health of plants for example, bacteria that normally live on plants and encourage the formation of ice crystals have been changed from frost - plus to frost - minus bacteria. Also, a bacterium that normally colonizes the roots of com plants has now been endowed with genes (from another bacterium) that code for an insect toxin. The toxin protects the roots from insects. Bacteria can be selected for their ability to degrade a particular substance and then this ability can be enhanced by genetic engineering. For instance, naturally occurring bacteria may be engineered to do an even better job of cleaning up beaches after oil spills.

Industry has found that bacteria can be used as biofilters to prevent airborne chemical pollutants from being vented into the air. They can also remove sulfur from coal before it is burned and help to clean up toxic waste dumps. One such strain was given genes that allowed it to clean up levels of toxins that would have killed other strains. Further, these bacteria were given “suicide” genes that caused them to self-destruct when the job had been accomplished.

Organic chemicals are often synthesized by having catalysts act on precursor molecules or by using bacteria to carry out the synthesis. Today, it is possible to go one step further and to manipulate the genes that code for these enzymes. For instance, biochemists discovered a strain of bacteria that is specially good at producing phenylalanine; an organic chemical needed to make aspartame, the dipeptide sweetener better known as Nutrasweet. They isolated, altered and formed a vector for the appropriate genes so that various bacteria could be genetically engineered to produce pucnylaianine. Many major mining companies already use bacteria to obtain various metals. Genetic engineering may enhance the ability of bacteria to extract copper, uranium and gold from low grade sources. Some mining companies are testing genetically engineered organisms that have improved bioleaching capabilities.

Animation 23.4: Transgenic Bectria
Source & Credit: 33rd Square

Transgenic Plants

Techniques have been developed to introduce foreign genes into immature plant embryos, or into plant cells that have had the cell wall removed and are called **protoplasts.** It is possible to treat protoplasts with an electric current while they are suspended in a liquid containing foreign DNA. The electric current makes tiny, selfscaling holes in the plasma membrane through which genetic material can enter. Then a protoplast will develop into a complete plant. Foreign genes transferred to cotton, com and potato strains have made these plants resistant to pests because their cells now produce an insect'toxin. Similarly, soybeans have been made resistant to a common herbicide. Some corn and cotton plants are both pest and herbicide resistant. In 1999 these transgenic crops were planted on more than 70 million acres worldwide and the acreage is expected to triple in about five years. Improvements still to come for are increased protein or starch content and modified oil or amino acid composition.

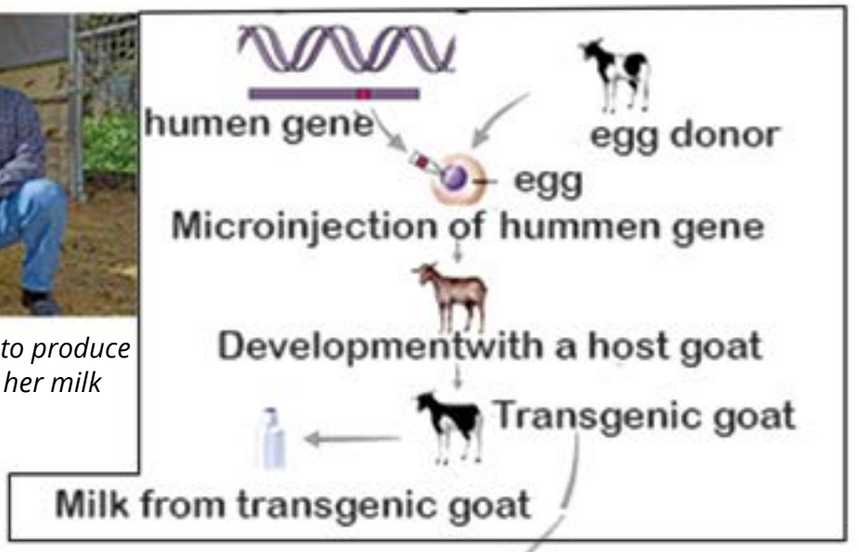
Animation 23.5:Transgenic Plants
Source & Credit: Wikipedia

Agribusiness companies also are in the process of developing transgenic versions of wheat and rice in addition to com. This is considered an absolute necessity if the 2020 global demand for rice, wheat and com is to be met. World grain harvests have continued to rise since the 1960s when special high-yield hybrid plants were developed during the so called green revolution. But the per capita production has now flattened out because of continued population growth. The hope is that genetic engineering will allow fanners to surpass the yield barrier. Perhaps, the stomata, the pore-like openings in the leaves, could be altered to boost carbon dioxide intake or cut down water loss.
Another possible goal is to increase the efficiency of the enzyme Rubisco which captures C02 in most plants. A team of Japanese scientists are attempting to introduce the C4 cycle into the rice. Plants that utilize the C4 cycle avoid the inefficiency of carboxylase by using a different means of capturing C02. Unlike the single gene transfers that have been done so far, these modifications would require a thorough re-engineering of plant cells. Single gene transfers will cause plants to produce various products. A weed called mouse-eared cress has been engineered to produce a biodegradable plastic (polyhydroxy-butyrate) in cell granules.
Plants are being engineered to produce human hormones, clotting factors, and antibodies in their seeds. One type of antibody made by com can deliver radio isotopes to tumor cells, and another made by soybeans can be used as treatment for genital herpes. Plant-made antibodies are inexpensive and there is little worry about contamination with pathogens that could infect people. Clinical trials have begun.

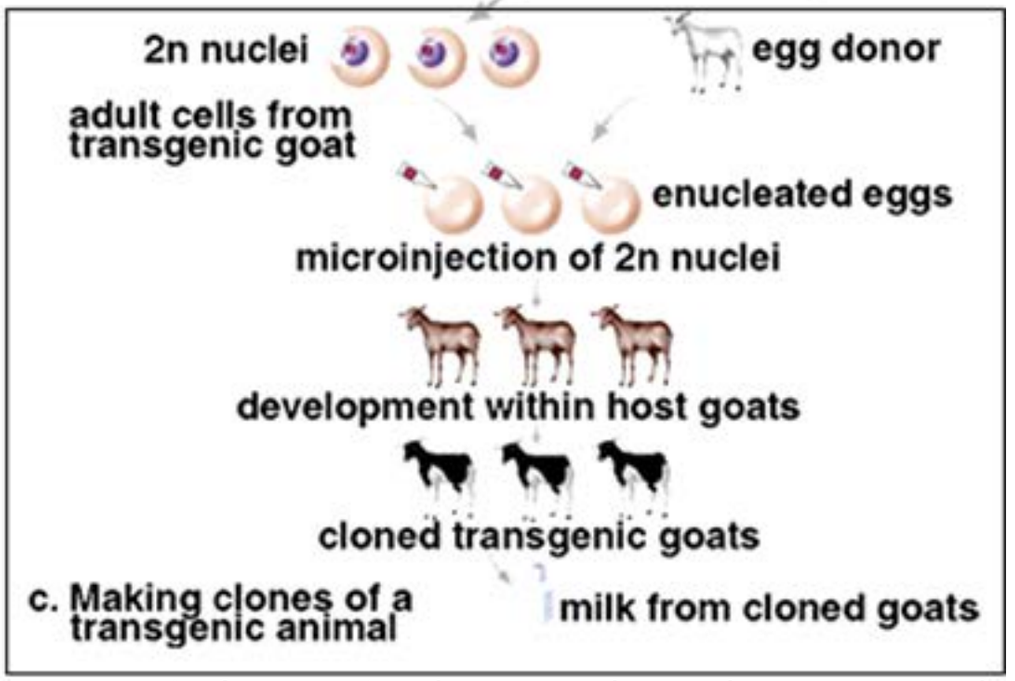
Transgenic Animals

Techniques have been developed to insert genes into the eggs of animals. It is possible to micro eggs by hand, but another method uses vortex mixing. The eggs and silicon - carbide needles, and the needles make DNA can enter. When these eggs are fertilized, the resulting offspring are transgenic animals. Using this technique many types of animal eggs have taken up the gene for bovine growth hormone. The procedure has been used to produce larger fishes', cows, pigs, rabbits and sheep. Genetically engineered fishes are now being kept in ponds that offer no escape to the wild because there is much concern that they will upset or destroy natural ecosystems.

(a) This goat is genetically engineered to produce antithrobin III , which is secreted in her milk



(b) The procedure to produce a transgenic animal.



(c) The procedure to clone a transgenic animal

Gene pharming, the' use of transgenic farm animals to produce pharmaceuticals is being pursued by a number of firms. Genes that code for therapeutic, and diagnostic proteins are incorporated into the animal's DNA, and the proteins appear in the animal's milk. There are plans to produce drugs for the treatment of cystic fibrosis, cancer, blood diseases and other disorders. Antithrombin III, for preventing blood clot during surgery, is currently being produced by a herd of goats, and clinical trials have begun. Figure 23.10 out lines the procedure of producing transgenic mammals. DNA containing the gene of interest is injected into donor eggs. Following in vitro fertilization, the zygotes are placed in host females where they develop. After female offspring mature, the product is secreted in the milk. The scientists of United States Department

of Agriculture have been able to genetically engineer mice to produce human growth hormone in their urine instead of in milk. They expect to be able to use the same technique on larger animals. Urine is a preferable vehicle for a biotechnology product than milk because all animals in a herd urinate - only females produce milk; animals start to urinate at birth - females don't produce milk until maturity; and its easier to extract proteins from urine than from milk.

Cloning of Transgenic Animals

Imagine that an animal has been genetically engineered to produce a biotechnology product. What would be the best possible method of getting identical copies of the animals? Asexual reproduction through cloning the animal would be the preferred procedure to use. Cloning is a form of asexual reproduction because it requires only the genes of that one animal. For many years it was believed that adult vertebrate animals could not be cloned. Although each cell contains a copy of all the genes certain genes are turned off in mature specialized cells. Different genes are expressed in muscle cells, which contract, compared to nerve cells, which conduct nerve impulses and to glandular cells, which secrete. Cloning of an adult vertebrate requires that all genes of an adult cells be turned on again if development is to proceed normally. It had long been thought this would be impossible. In 1997, scientists at the Roslin Institute in Scotland announced that they achieved this feat and had produced a cloned sheep called Dolly.

Since then calves and goats have been cloned. Figure 23.10 shows that after enucleated eggs have been injected with 2n nuclei of adult cells, they can be coaxed to begin development. The offspring have the genotype and phenotype of the adult that donated the nuclei; therefore, the adult has been cloned. In the procedure that produced cloned mice, the 2n nuclei were taken from cumulus cells.

Cumulus cells are those that cling to an egg after ovulation occurs. A specially prepared chemical bath was used to stimulate the eggs to divide and begin development. Now that scientists have a method to clone mammals, this procedure will undoubtedly be used routinely. In the United States, a presidential order prohibits the cloning of humans. But certain other countries are experimenting with the possibility.

GENE THERAPY

Gene therapy is the insertion of genetic material into human cells for the treatment of a disorder. It includes procedures that give a patient healthy genes to make up for faulty genes and also includes the use of genes to treat various other human illnesses such as cancer and cardiovascular diseases.

Animation 23.6: Gene Therapy
Source & Credit: Ethris

There are two main methods used for gene therapy Ex-vivo and in vivo. Ex- vivo gene therapy is shown in Fig. 23.11. in which children in the severe combined immunodeficiency syndrome (SCID) is treated. These children lack an enzyme adenosine deaminase (ADA) that is involved in the maturation of T and B cells and, therefore, they are subjected to life threatening infections. Bone marrow stem cells are removed from the blood and infected with a retrovirus (RNA virus) that carries a normal gene for the enzyme then the cells are returned to the patient. Bone marrow stem cells are preferred for this procedure, because they divide to produce more cells with same genes. Patients who have undergone this procedure do have a significant improvement in their immune function that is associated with a sustained rise in the level of ADA enzyme activity in the blood

Among the many gene therapy trials, one is for the treatment of familial hypercholesterolemia a condition that develops when liver cells lack a receptor for removing cholesterol from the blood. The high levels of blood cholesterol make the patient subject to fatal heart attacks at a young age. In a newly developed procedure, a small portion of the liver is surgically excised and infected with a retrovirus containing a normal gene for the receptor. Several patients have experienced a lowering of serum cholesterol levels following this procedure.

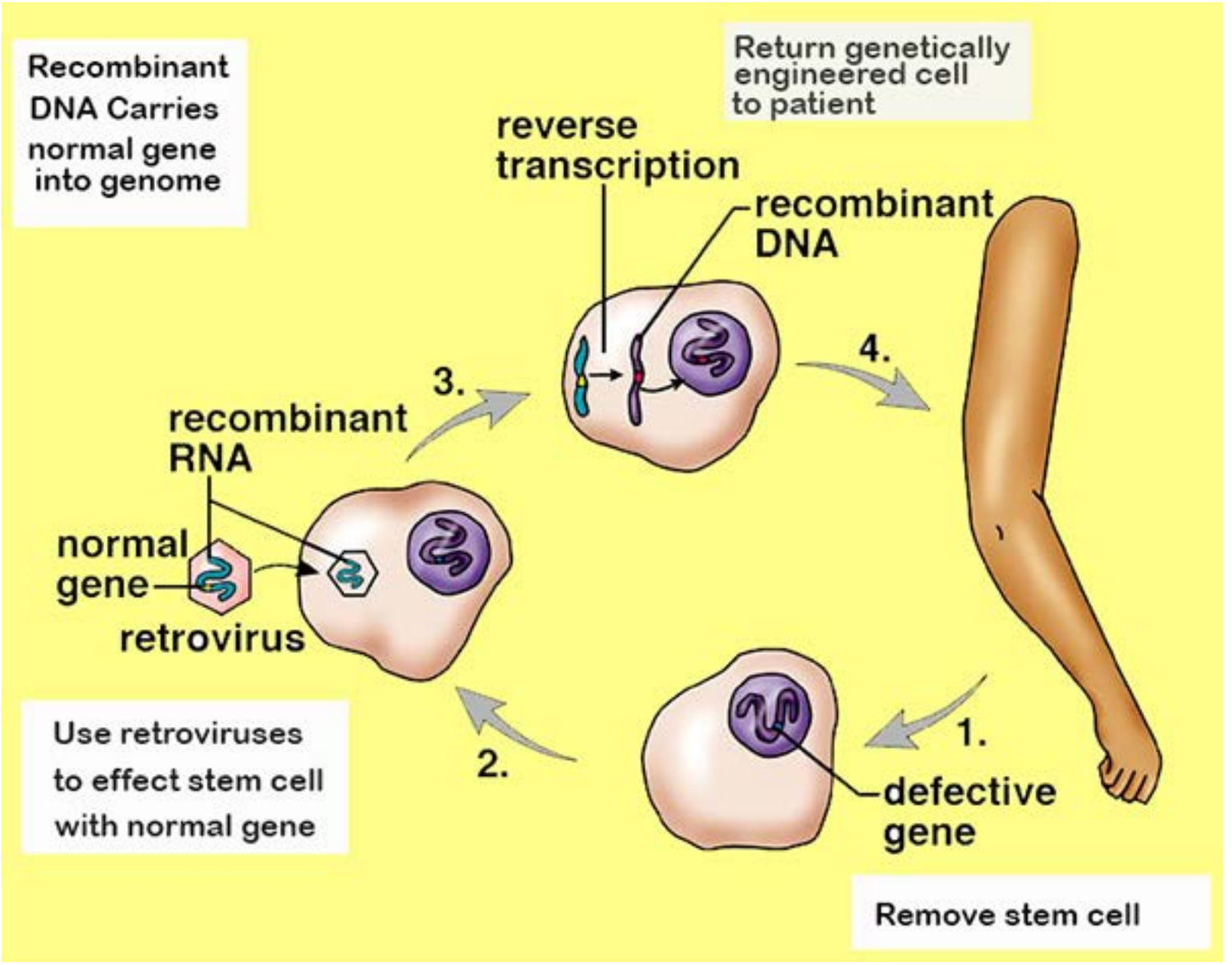


Fig 23.11 Ex vivo gene therapy in human

Cystic fibrosis patients lack a gene that codes for trans-membrane carrier of the chloride ion. Patients often die due to numerous infections of the respiratory tract. And in vivo method of treatment is being tried. Liposomes-microscopic vesicles that spontaneously form when lipoproteins are put into a solution, have been coated with the gene needed to cure cystic fibrosis. Then the solution is sprayed into patient's nostrils. Due to limited gene transfer, this methodology has not as yet been successful.

Gene therapy is also being done to cancer patients, which makes them more tolerant of chemotherapy. In clinical trials researchers have given genes to cancer patient that either make healthy cells more tolerant of chemotherapy or make tumors more vulnerable to it. Once the bone marrow stem cells were protected it was possible to increase the level of chemotherapy to kill the cancer cells.

During coronary artery angioplasty, a balloon catheter is sometimes used to open up a closed artery. Unfortunately, the artery has a tendency to close up once again. But investigators have come up with a new procedure. The balloon is coated with a plasmid that contains a gene for vascular endothelial growth factor. The expression of the gene, which promotes the proliferation of blood vessels to bypass the obstructed area, has been observed in at least one patient.

Perhaps it will be possible to used in vivo therapy to cure hemophilia, diabetes. Parkinson disease, or AIDS. To treat hemophilia, patients could get regular doses of cells that contain normal clotting-factor genes or such cells could be placed in organoids, artificial organs that can be implanted in the abdominal cavity. To cure Parkinson's disease, dopamine-producing cells could be grafted directly into the brain.

TISSUE CULTURE

Tissue culture is the growth of a tissue in an artificial liquid culture medium. German botanist Gottlieb Haberlandt said in 1902 that plant cells are totipotent - each cell has the full genetic potential of the organism - and, therefore, a single cell could become a complete plant. But it wasn't until 1958 that Cornell botanist F.C. Steward grew a complete carrot plant from a tiny piece of phloem. He provided the cells with sugars, minerals and vitamins, but he also added coconut milk. (Later it was discovered that coconut milk contains the plant hormone cytokinin). When the cultured cells began dividing, they produced a callus, an undifferentiated group of cells.

Then the callus differentiated into shoot and roots and developed into a complete plant.

Tissue culture techniques have by now led to micropropagation, a commercial method of producing thousands, even millions of identical seedlings in a limited amount of space. One favourite method to accomplish micro propagation is by meristem culture. If the correct proportions of auxins and cytokinin are added to a liquid medium, many new shoots will develop from a single shoot tip. When these are removed more shoots form. Since the shoots are genetically identical the adult plants that develop from them are called clonal plants, all having the same traits. Another advantage of meristem culture is that meristem, unlike other portions of a plant, is virus free, therefore the plants produced are also virus free (The presence of plant viruses weakens plants and makes them less productive).

Because plants are totipotent, it should be possible to grow an entire plant from a single cell. This, too has been done. Enzymes are used to digest the cell walls of a small piece of tissue, usually mesophyll tissue, from a leaf, and the result is naked cells without walls, called protoplasts. The protoplasts regenerate a new cell wall and begin to divide. These clumps of cells can be manipulated to produce somatic embiyos. Somatic embryos that are encapsulated in a protective hydrated gel (and sometimes called artificial seeds) can be shipped everywhere. It is possible to produce millions of somatic embryos at once in large tanks called bioreactors. This is done for certain vegetables like tomato, celery, asparagus and for ornamental plants like lilies, begonias and African violets. A mature plant develops from each somatic embryo. Plants generated from the somatic embryo vary somewhat because of mutations that arise; dunng the production process. These so called somaclonal variations are another way to produce new plants with desired traits.

Anther culture is a technique in which mature anthers are cultured in a medium containing vitamins and growth regulators. The haploid tube cells with in the pollen grains divide, producing proembryos consisting of as many as 20 to 40 cells. Finally the pollen grains rupture releasing haploid embryos. The experimenter can now generate a haploid plant, or chemical agent can be added that encourages chromosomal doubling. After chromosomal doubling the resulting plants are diploid but homozygous for all their alleles. Anther culture is a direct way to produce plants that express recessive alleles. If the recessive alleles govern desirable traits, the plants have these traits.

The culturing of plant tissues has led to a technique called cell suspension culture. Rapidly growing cultures are cut into small pieces and shaken in'a liquid nutrient medium so that single cells or small clumps of cells break off and form a suspension. These cells will produce the same chemicals as the entire plant. For example cell suspension cultures of Cinchona ledgeriana produce quinine and those of Digitalis lanata produce digitoxin. Scientists envision that it will be possible to maintain cell suspension cultures in bioreactors for the purpose of producing chemicals used in the production of drugs, cosmetics and agricultural chemicals. If so, it will no longer be necessary to farm plants for the purpose of acquiring the chemicals they produce.

Genetic Engineering of Plants

Traditionally, hybridization, the crossing of different varieties of plants or even species, was used to produce plants with desirable traits. Hybridization, followed by vegetative propagation of the mature plants, generated a large number of identical plants with these traits. Today it is possible to directly alter the genes of organisms. Transgenic plants carry a foreign gene that has been introduced into their cells so that they have new and different traits.

Since a whole plant will grow from a protoplast, it is necessary only to place the foreign gene into a living- protoplast. A foreign gene isolated from any type of organism is placed in the tissue culture medium.

High-voltage electric pulses can then be used to create pores in the plasma membrane so that the DNA enters. In one of the first procedures carried out, a gene for the production of the firefly enzyme luciferase was inserted into tobacco protoplast and the adult plants glowed when sprayed with the substrate luciferin (Fig. 23.12).

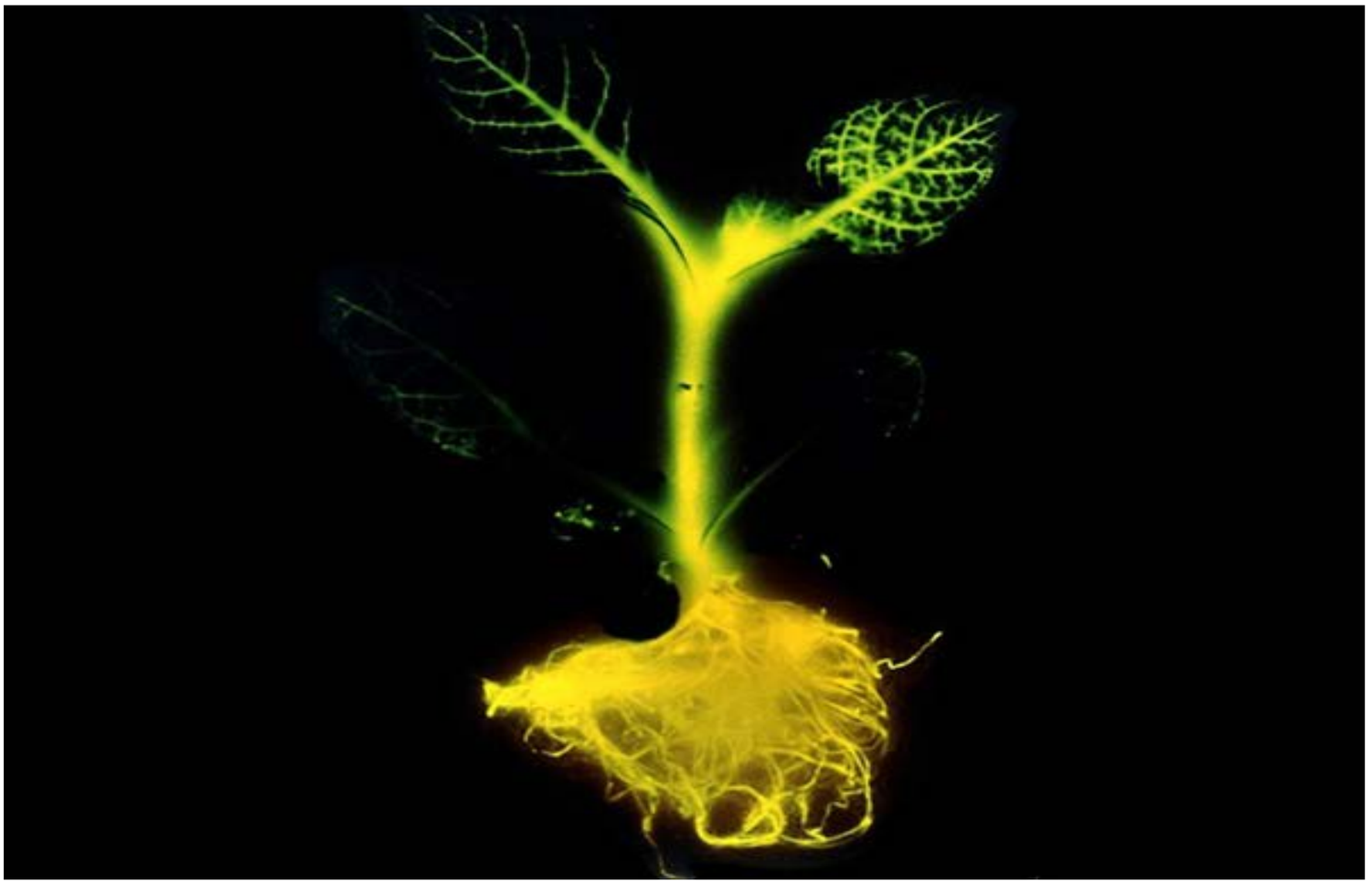


Fig 23.12 Tabacoo plant containing Luciferase gene glows when sprayed with luciferin

Unfortunately, the regeneration of cereal grains from protoplasts has been difficult. Com and wheat protoplasts produce infertile plants. As a result, other methods are used to introduce DNA into plant cells with intact cell wall. In one technique, foreign DNA is inserted into the plasmid of the bacterium *Agrobacterium,* which normally infects the plant cells. A plasmid can be used to produce' recombinant DNA. Recombinant DNA contains genes from different sources, namely those of plasmids and the foreign genes of interest. When the bacterium infects the plant the recombinant plasmid is introduced into the plant cells (Fig.23.12). In 1987, John C Sanford and Theodore M. Klein of Cornell University developed another method of introducing DNA into a plant tissue culture callus.

They constructed a device, called the particle gun, that bombards a callus with DNA coated microscopic metal particles. Then genetically altered somatic embryos develop into genetically adult plants. Many plants including com and wheat varieties have been genetically engineered by this method.

Agricultural Plants with Improved Traits

Cotton, com, potato and soybean plants have been engineered to be resistant to either insect predation or herbicides that are judged to be environmentally safe. Some com and cotton plants have been produced that are both insect and herbicide resistant. In 1999, transgenic crops were planted on more that 70 million acres world wide and the acreage is expected to triple in about five years. If crops are resistant to a broad-spectrum herbicide and weeds are not then the herbicide can be used to kill the weeds. When herbicide resistant plants were planted weeds were easily controlled, less tillage was needed and soil erosion was minimized.

One aim of genetic engineering is to produce crops that have the improved agricultural or food quality traits such as those listed in the table below:

Improved Agricultural Traits	
Herbicide resistant	Wheat, rice, sugar beets, canola
Salt tolerant	Cereals, rice, sugarcane
Drought tolerant	Cereals, rice, sugarcane
Cold tolerant	Cereals, rice, sugarcane
Improved yield	Cereals, rice, com, cotton
Modified wood pulp	Trees

Improved Food Quality Traits	
Fatty acid / oil content	Com, soybeans
Protein / starch content	Cereals, potatoes, soybeans, rice, com
Amino acid content	Com, soybean
Disease protected	Wheat, com, potatoes

Production of salt tolerant plants had been a dream of genetic engineer. Recently salt - tolerant *Arabidopsis* has been produced. For this the scientists first identified a gene coding for a channel protein that transports Na+ along with H+ across a vacuole membrane. Isolating Na+ in a vacuole prevents it from interfering with plant metabolism. Then, the scientists cloned the gene and used it to genetically engineer plants that overproduce the channel protein. The modified plants thrived when watered with a salty solution. Irrigation, even into fresh water, inevitably leads to a salinization of soil that reduces crop yields. Today, crop production is limited by effects of salinization at about 50% of irrigated levels. The next step to solve this problem is to produce salt - tolerant crops. It is believed that the production not only of salt - but also drought and cold tolerant crops will reduce the need for added farm acreage by increasing agricultural yields that will provide enough food for a world population that is expected to nearly double by 2050.

Some progress has also been made to increase the food quality of crops. Soybeans have been developed that mainly produce the monounsaturated fatty acid, oleic acid, a change that may improve human health. These altered plants also produce vernolic acid and ricinoleic acid, derivatives of oleic acid that can be used as hardenes in paints and plastics. The necessary genes were derived from Vemonia and castor bean seeds and were transferred into the soybean genomes.

Genetic Engineering is also expected to increase productivity. To that end, stomata might be altered to boost carbon dioxide intake or cut down water loss. The efficiency of the enzyme RuBP carboxylase which captures C02 in plants could be improved. A team of Japanese scientists is working on introduc ing the C4 photosynthetic cycle into rice. Unlike C3 plants, C4 plants do well in hot dry weather. These modifications would require a more complete engineering of plant cells than the single gene transfers' that have been done so far.

Production of Products

Single gene transfers have allowed plants to produce various products such as human hormones, clotting factors and antibodies. One type of antibody made by com can deliver radioisotopes to tumor cells and another made by soybeans can be used as treatment for genital herpes clinical triats have begun.

Recently, a group of scientists from Biosource Technologies located in Vacaville, California reported that they have been able to use the tobacco mosaic virus as a vector to introduce a human gene into adult tobacco plants in the field. Note that this technology by passes the need for tissue culture completely. Tens of grams of a-galactosidase, an enzyme that can be used to treat a human lysosome storage disease, were harvested per acre of tobacco plants. And it only took thirty days to get tobacco plants to produce antigens to treat non-Hodgkin's lymphoma after being sprayed with a genetically engineered vims.

EXERCISE

Q.1. Fill in the blanks.

1. The use of polymerase chain reaction (PCR) creates a _____ of copies in a laboratory test tube.
2. _____free living organisms in the environment that have had a foreign gene inserted into them.
3. _____known sequences of DNA that are used to find complementary DNA strands; can be used diagnostically to determine the presence of particular gene.
4. _____production of many identical copies of a gene.
5. _____self duplicating ring of accessory DNA in the cytoplasm of bacteria.

Q.3. Short questions.

1. How and why transgenic animals that secrete a product are often cloned?
2. Explain two primary goals of Human Genome Project. What are possible benefits of the project?
3. Explain and give examples of ex vivo and in vivo gene therapies in humans?

Q.4. Extensive questions.

1. What is the methodology for producing recombinant DNA to be used in gene cloning?
2. What is a genomic library, how would you locate a gene^of.interest in the library?
3. What is the polymerase chain reaction (PCR), amfftow is it carried out to produce multiple copies of a DNA segment?
4. What is DNA finger printing, a process that utilizes the entire genome?
5. For what purpose have bacteria, plants and animals been genetically altered?-