Recombinant DNA technology

DSE2

Compiled by

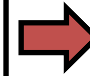
Anwesha Bandyopadhyay

Department of Botany
Bidhan Chandra College

What is Biotechnology?

- **Biotechnology involves the use of living organisms in industrial processes—particularly in agriculture, food processing, and medicine.**
- **Biotechnology has been around since the dawn of time, ever since humans began manipulating the natural environment to improve their food supply, housing, and health**

Traditional Biotechnology Products
Bread, cheese, wine, and beer have been made worldwide for many centuries using microorganisms, such as yeast.



Recombinant DNA: A single DNA molecule made from two different sources,

Making Multiple Copies of a Gene or Other DNA Segment

❑ **DNA cloning is the process which is involved in preparing well defined segment of DNA (gene) in multiple identical copies.**

Why cloning?

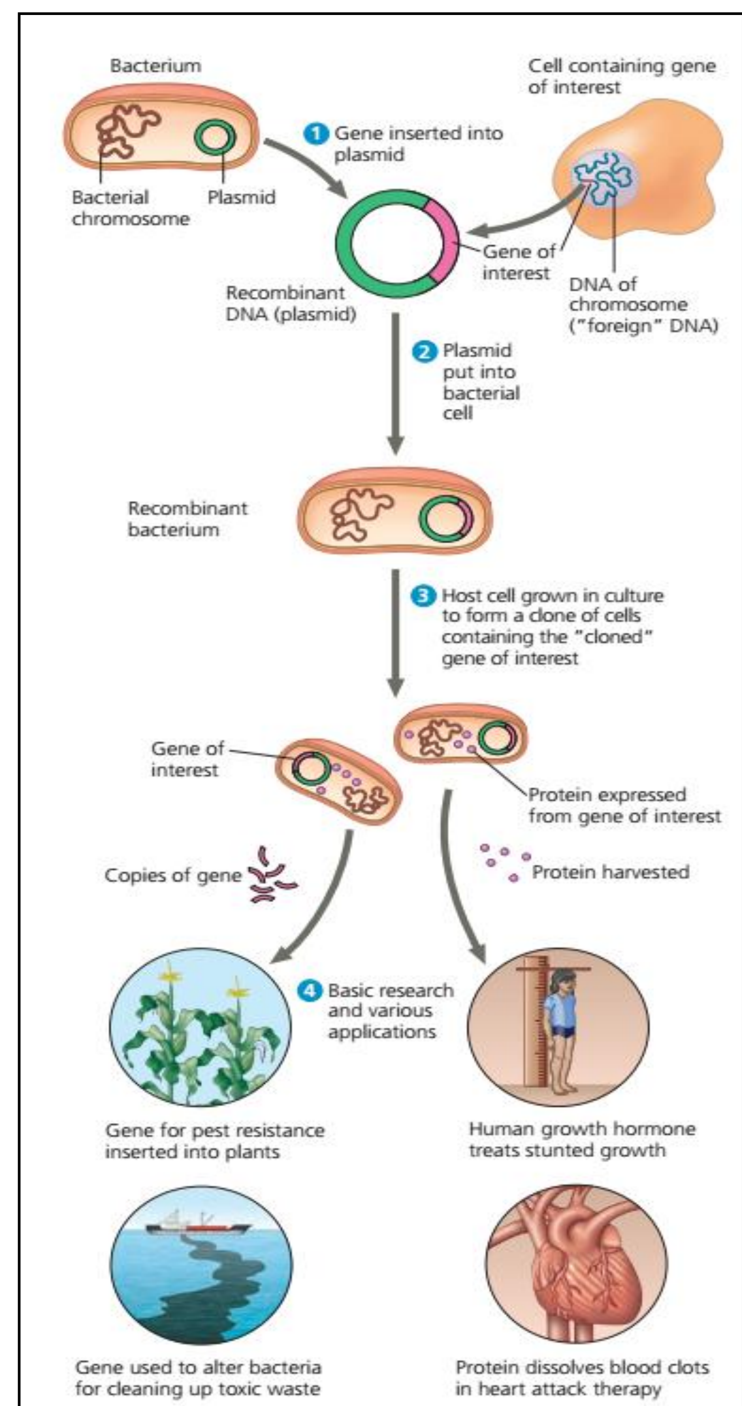
- Generally, molecular biologists work with a single or particular gene
- Naturally occurring DNA molecules are very long, and a single molecule usually carries many hundreds or even thousands of genes.
- In many eukaryotic genomes, protein-coding genes occupy only a small proportion of the chromosomal DNA, the rest being non-coding nucleotide sequences.

Gene cloning is useful for two basic purposes: to make many copies of, or *amplify*, a particular gene and to produce a protein product. Researchers can isolate copies of a cloned gene from bacteria for use in basic research or to endow another organism with a new metabolic capability, such as pest resistance. For example, a resistance gene present in one crop species might be cloned and transferred into plants of another species. Alternatively, a protein with medical uses, such as human growth hormone, can be harvested in large quantities from cultures of bacteria carrying a cloned gene for the protein. (We'll describe the techniques for expressing cloned genes later.) Since one gene is only a very small part of the total DNA in a cell, the ability to amplify such rare DNA fragments, by cloning or other means, is crucial for any application involving a single gene

Most methods for cloning pieces of DNA in the laboratory share certain general features. One common approach uses bacteria, most often *Escherichia coli*. *E. coli* chromosome is a large circular molecule of DNA. In addition to their bacterial chromosome, *E. coli* and many other bacteria also have **plasmids**, small, circular DNA molecules that are replicated separately. A plasmid has only a small number of genes; these genes may be useful when the bacterium is in a particular environment but may not be required for survival or reproduction under most conditions

To clone pieces of DNA using bacteria, researchers first obtain a plasmid (originally isolated from a bacterial cell and genetically engineered for efficient cloning) and insert DNA from another source ("foreign" DNA) into it **(See Figure)**. The resulting plasmid is now a **recombinant DNA** molecule, a molecule containing DNA from two different sources, very often different species. The plasmid is then returned to a bacterial cell, producing a *recombinant bacterium*. This single cell reproduces through repeated cell divisions to form a clone of cells, a population of genetically identical cells. Because the dividing bacteria replicate the recombinant plasmid and pass it on to their descendants, the foreign DNA and any genes it carries are cloned at the same time. The production of multiple copies of a single gene is a type of DNA cloning called **gene cloning**.

In our example in the Figure, the plasmid acts as a **cloning vector**, a DNA molecule that can carry foreign DNA into a host cell and replicate there. Bacterial plasmids are widely used as cloning vectors for several reasons: They can be readily obtained from commercial suppliers, manipulated to form recombinant plasmids by insertion of foreign DNA in a test tube (*in vitro*, from the Latin meaning "in glass"), and then easily introduced into bacterial cells. Moreover, recombinant bacterial plasmids (and the foreign DNA they carry) multiply rapidly owing to the high reproductive rate of their host (bacterial) cells. The foreign DNA in Figure 20.5 is a gene from a eukaryotic cell; we will describe in more detail how the foreign DNA segment was obtained later in this section



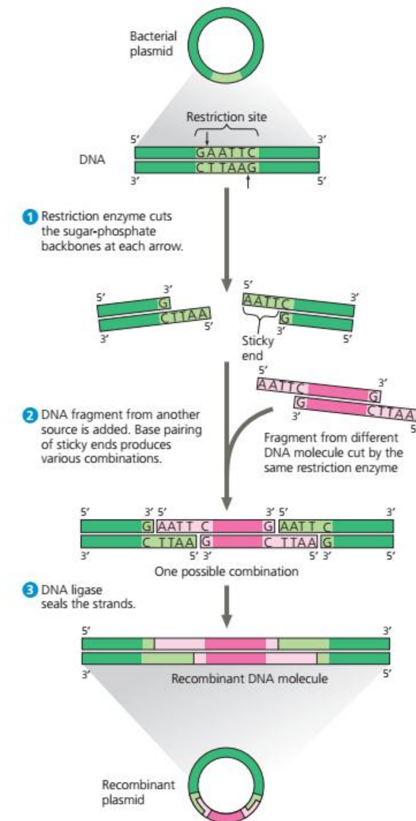
Restriction enzymes

❖Enzymes that cut DNA molecules at a limited number of specific locations. These bacterial enzymes bind to specific recognition sites on DNA and cut the backbone of both strands.

❖Restriction enzymes have been exploited to cut DNA at specific sites, since each restriction enzyme has a particular recognition sequence.

❖ On the basis of differences in cleavage site restriction enzymes are of two types

Type I restriction enzymes cut the DNA strand 1000 or more base pairs from the recognition sequence.
Type II restriction enzymes cut in the middle of the recognition sequence and are the most useful for genetic engineering



5′- GTTAAC -3′
3′- CAATTG -5′

CUT BY *Hpa*I

5′- GTT AAC -3′
3′- CAA TTG -5′

BLUNT ENDS

5′- GAATTC -3′
3′- CTTAAG -5′

CUT BY *Eco*R1

AATTC -3′
G -5′
5′- G
3′- CTTAA

STICKY ENDS

Type II restriction enzymes can either cut both strands of the double helix at the same point, leaving **blunt ends**, or they can cut at different sites on each strand leaving single stranded ends, sometimes called **sticky ends**

Table 3.1 Table of Common Restriction Enzymes

Enzyme	Source Organism	Recognition Sequence
*Hpa*II	*Haemophilus parainfluenzae*	C/CGG GGC/C
*Mbo*I	*Moraxella bovis*	/GATC GATC/
*Nde*II	*Neisseria denitrificans*	/GATC GATC/
*Eco*RI	*Escherichia coli* RY13	G/AATTC CTTAA/G
*Eco*RII	*Escherichia coli* RY13	/CCWGG GGWCC/
*Eco*RV	*Escherichia coli* J62/pGL74	GAT/ATC CTA/TAG
*Bam*HI	*Bacillus amyloliquefaciens*	G/GATCC CCTAG/G
*Sau*I	*Staphylococcus aureus*	CC/TNAGG GGANT/CC
*Bgl*I	*Bacillus globigii*	GCCNNNN/NGGC CGGN/NNNNCCG
*Not*I	*Nocardia otitidis-caviarum*	GC/GGCCGC CGCCGG/CG
*Dra*II	*Deinococcus radiophilus*	RG/GNCCY YCCNG/GR

/, position where enzyme cuts.
N, any base; R, any purine; Y, any pyrimidine; W, A or T.

When two different DNA samples are cut with the same sticky-end restriction enzyme, all the fragments will have identical overhangs. This allows DNA fragments from two sources (e.g., two different organisms) to be linked together. Fragments are linked or **ligated** using **DNA ligase**, the same enzyme that ligates the Okazaki fragments during replication. The most common ligase used is actually from T4 bacteriophage. Ligase catalyzes linkage between the 3′-OH of one strand and the 5′-PO4 of the other DNA strand. Ligase is much more efficient with overhanging sticky ends, but can also link blunt ends much more slowly.

Applications of Biotechnology

Agricultural Applications

Scientists are working to learn more about the genomes of agriculturally important plants and animals. For a number of years, they have been using DNA technology in an effort to improve agricultural productivity. The selective breeding of both livestock (animal husbandry) and crops has exploited naturally occurring mutations and genetic recombination for thousands of years. Agricultural scientists have already endowed a number of crop plants with genes for desirable traits, such as delayed ripening and resistance to spoilage and disease, as well as drought. The most commonly used vector for introducing new genes into plant cells is a plasmid, called the *Ti plasmid*, from the soil bacterium *Agrobacterium tumefaciens*. This plasmid integrates a segment of its DNA into the chromosomal DNA of its host plant cells. To make transgenic plants, researchers engineer the plasmid to carry genes of interest and introduce it into cells. For many plant species, a single tissue cell grown in culture can give rise to an adult plant. Thus, genetic manipulations can be performed on an ordinary somatic cell and the cell then used to generate an organism with new traits.

Genetic engineering is rapidly replacing traditional plant breeding programs, especially for useful traits, such as herbicide or pest resistance, determined by one or a few genes. Crops engineered with a bacterial gene making the plants resistant to an herbicide can grow while weeds are destroyed, and genetically engineered crops that can resist destructive insects reduce the need for chemical insecticides. In India, the insertion of a salinity resistance gene from a coastal mangrove plant into the genomes of several rice varieties has resulted in rice plants that can grow in water three times as salty as seawater. The research foundation that carried out this feat of genetic engineering estimates that one-third of all irrigated land has high salinity owing to over irrigation and intensive use of chemical fertilizers, representing a serious threat to the food supply. Thus, salinity-resistant crop plants would be enormously valuable worldwide.

Biotechnology Applications in Agriculture

1. The use of **Biotechnology** in **Agriculture** is known as **Green Biotechnology**. Biotechnology had contributed a lot towards the upliftment of agriculture. The organisms formed after manipulation of genes is known as **Genetically Modified Organisms (GMOs)** such as Crops, Animals, Plants, Fungi, Bacteria etc. Genetically modified crops are formed by manipulation of **DNA** to introduce new trait into the crops. These manipulations are done to introduce traits such as pest resistance, insect resistance, weed resistance etc.

Bt. Cotton. ***Bacillus thuringiensis*** is a bacterium that produces an endotoxin which is insecticidal. This toxin when taken in by an insect, the insect get paralyzed and dies off. The gene responsible for producing endotoxin is isolated and inserted into the genome of the cotton plant. This prevents the cotton plant from the attack of the insects.

A nematode known as ***Melodegyne incognitia*** infects the roots to the tobacco plant. This reduces the yield of the plant. To prevent this, **RNA** interference strategy is used. This uses a complementary **RNA** that degrades the **mRNA** of nematode responsible for infection in roots of the tobacco plant.

2. Distant Hybridization:

With the advancement of genetic engineering, it is now possible to transfer genes between distantly related species. The barriers of gene transfer between species or even genera have been overcome. The desirable genes can be transferred even from lower organisms to higher organisms through recombinant DNA technology.

3. Development of Root Nodules in Cereal Crops:

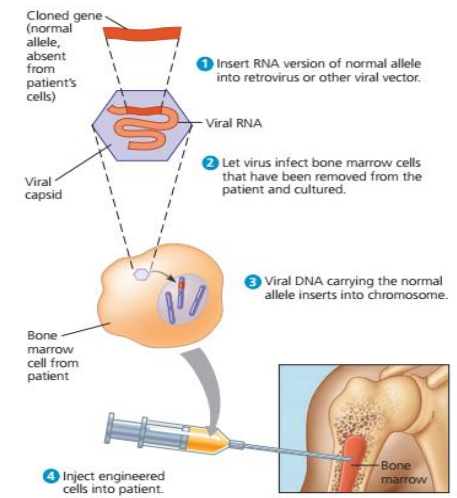
Leguminous plants have root-nodules which contain nitrogen fixing bacteria Rhizobium. This bacteria converts the free atmospheric nitrogen into nitrates in the root nodules. The bacterial genes responsible for this nitrogen fixation can be transferred now to cereal crops like wheat, rice, maize, barley etc. through the techniques of genetic engineering thus making these crops too capable of fixing atmospheric nitrogen.

4. Development of C_4 Plants:

Improvement in yield can be achieved by improving the photosynthetic efficiency of crop plants. The photosynthetic rate can be increased by conversion of C_3 plants into C_4 plants, which can be achieved either through protoplasm fusion or recombinant DNA technology C_4 plants have higher potential rate of biomass production than C_3 plants. Most C_4 plants (sorghum, sugarcane, maize, some grasses) are grown in tropical and subtropical zones.

Human Gene Therapy

Gene therapy—the introduction of genes into an afflicted individual for therapeutic purposes—holds great potential for treating the relatively small number of disorders traceable to a single defective gene. In theory, a normal allele of the defective gene could be inserted into the somatic cells of the tissue affected by the disorder For gene therapy of somatic cells to be permanent, the cells that receive the normal allele must be cells that multiply throughout the patient's life. Bone marrow cells, which include the stem cells that give rise to all the cells of the blood and immune system, are prime candidates. The **Figure in the right hand side** outlines one procedure for gene therapy of an individual whose bone marrow cells do not produce a vital enzyme because of a single defective gene. One type of severe combined immunodeficiency (SCID) is caused by this kind of defect. If the treatment is successful, the patient's bone marrow cells will begin producing the missing protein, and the patient may be cured.



▲ **Figure 20.22 Gene therapy using a retroviral vector.** A retrovirus that has been rendered harmless is used as a vector in this procedure, which exploits the ability of a retrovirus to insert a DNA transcript of its RNA genome into the chromosomal DNA of its host cell (see Figure 19.8). If the foreign gene carried by the retroviral vector is expressed, the cell and its descendants will possess the gene product. Cells that reproduce throughout life, such as bone marrow cells, are ideal candidates for gene therapy.

Biotechnology Applications in Medicine

The use of biotechnology in medicine is known as **Medicinal Biotechnology**. This helps in formation of genetically modified insulin known as humulin. This helps in treatment of large number of diabetes patients.
Biotechnology has given rise to a technique known as gene therapy. Gene therapy is a technique to remove genetic defect in embryo or child. This technique involves transfer of a normal gene that works over non-functional gene.

Biotechnology in Diagnosis

It helps in disease diagnosis by various techniques such as **ELISA**, **PCR** etc. **ELISA** (Enzyme Linked Immunosorbent Assay) is based on antigen and antibody reaction to detect different diseases. **PCR** (Polymerase Chain Reaction) is technique to amplify specific **DNA** segment. This helps to detect **HIV** in **AIDS** patients.

Biopharmaceutical Drug Development:

In the field of biopharmaceutical drug development, it is the development of therapeutic human proteins by recombinant methods. (Table 22.5) for use as medicines that has the longest tradition. As mentioned above, recombinant human insulin was the first recombinant medicine in the world, produced by Genentech and brought to market in 1982. Today, recombinant human insulin has almost completely driven the other preparation of insulin (isolated from human or animal tissues) from the market.

Table 22.5. Selected examples of recombinant proteins with indication and manufacturer.

Drug	Product name	Indication	Manufacturer
Human insulin	Humulin	Diabetes mellitus type I	Eli Lilly
Somatotropin	Humatrope	Inadequate growth	Eli Lilly
Erythropoietin alpha	Erypo/Epogen	Anemia	Jansen-Cilag/Amgen
Factor VIII	Bioclate/Kogenate	Hemophilia	Centeon/Bayer
Interferon alpha 2 a	Roferon A	Cancer	Roche
Interferon beta 1 b	Betaferon	Mulitples sclerosis	Schering
Tissue plasminogen activator tPA (alteplase)	Actilyse	Thrombolytic agent	Boehringer Ingelheim

The first therapeutic antibodies, especially monoclonal antibodies, have been on the market since the late 1990s. In 2002, antibodies were (along with vaccines) the most important therapeutic class of drugs under development and there are also more recent market studies more than 100 antibodies or antibody fragments were at the clinical development stage in 2002 and research and development is being carried out on around 470 more in about 200 companies around the world (Table 22.6,7).

Table 22.6. Selected examples of approved monoclonal antibodies.

Drug	Product name	Indication	Manufacturer
Abciximab Centocor Europe	Reopro	Anticoagulant	Eli Lilly
Trastuzumab (anti-HERA2-a)	Herceptin	Breast cancer	Roche
Adalimumab (anti-TNF-alpha)	Humira	Rheumatoid arthritis	Abbott
Infliximab (anti-TNF-alpha)	Remicade	Crohn's disease	Centocor
Alematuzumab (anti CD52)	Campth	Leukemia	Millennium &Ilex

Table 22.7. Selected examples of therapeutic RNAs on the market or under development.

Principle of action	Product name/production stage	Indication	Company
Antisense	Vitravene/market	CMV retinitis	ISIS pharmaceuticals
Antisense	Affinitak/phase II	Cancer	ISIS pharmaceuticals
Antisense	Alicaforsen/phase III	Crohn's disease	ISIS pharmaceuticals
Antisense	AP 12009/phase II	Brain tumor	Antisense pharma
Ribozyme	ANGIOZYME/phase II	Intestinal cancer	Sirna therapeutics

Environmental Cleanup

Increasingly, the remarkable ability of certain microorganisms to transform chemicals is being exploited for environmental cleanup. If the growth needs of such microbes make them unsuitable for direct use, scientists can now transfer the genes for their valuable metabolic capabilities into other microorganisms, which can then be used to treat environmental problems. For example, many bacteria can extract heavy metals, such as copper, lead, and nickel, from their environments and incorporate the metals into compounds such as copper sulfate or lead sulfate, which are readily recoverable. Genetically engineered microbes may become important in both mining (especially as ore reserves are depleted) and cleaning up highly toxic mining wastes.

Biotechnologists are also trying to engineer microbes that can degrade chlorinated hydrocarbons and other harmful compounds. These microbes could be used in wastewater treatment plants or by manufacturers before the compounds are ever released into the environment.