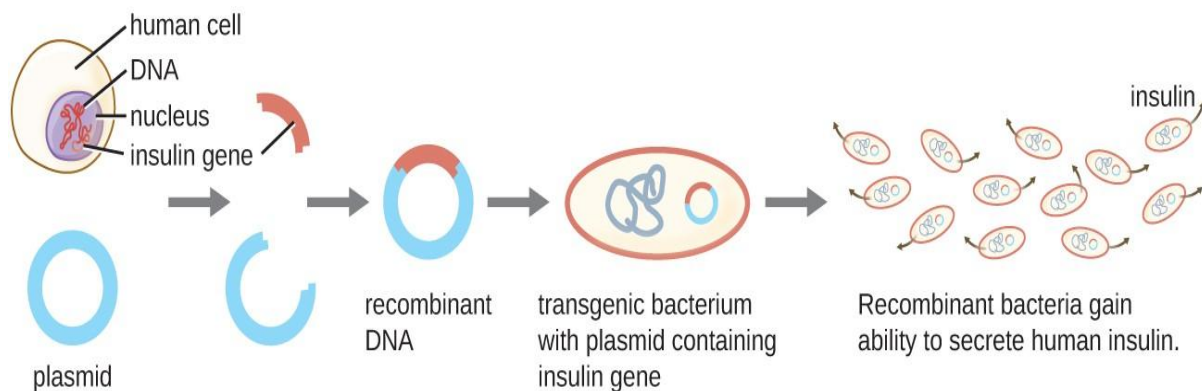


Tools in Genetic engineering

The science of using living systems to benefit humankind is called **biotechnology**. Technically speaking, the domestication of plants and animals through farming and **breeding** practices is a type of biotechnology. However, in a contemporary sense, we associate biotechnology with the direct alteration of an organism's genetics to achieve desirable traits through the process of **genetic engineering**.

Genetic engineering involves the use of **recombinant DNA technology**, the process by which a DNA sequence is manipulated *in vitro*, thus creating **recombinant DNA molecules** that have new combinations of genetic material. The recombinant DNA is then introduced into a host organism. If the DNA that is introduced comes from a different species, the host organism is now considered to be **transgenic**.

One example of a transgenic microorganism is the bacterial strain that produces human **insulin**. The insulin gene from humans was inserted into a plasmid. This recombinant DNA plasmid was then inserted into bacteria. As a result, these transgenic microbes are able to produce and secrete human insulin. Many prokaryotes are able to acquire foreign DNA and incorporate functional genes into their own genome through “mating” with other cells (**conjugation**), viral infection (**transduction**), and taking up DNA from the environment (**transformation**). Recall that these mechanisms are examples of **horizontal gene transfer**—the transfer of genetic material between cells of the same generation.



Molecular Cloning

Herbert **Boyer** and Stanley **Cohen** first demonstrated the complete **molecular cloning** process in 1973 when they successfully cloned genes from the African clawed frog (*Xenopus laevis*) into a bacterial plasmid that was then introduced into the bacterial host *Escherichia coli*. Molecular cloning is a set of methods used to construct recombinant DNA and incorporate it into a host organism; it makes use of a number of molecular tools that are derived from microorganisms.

Restriction Enzymes and Ligases

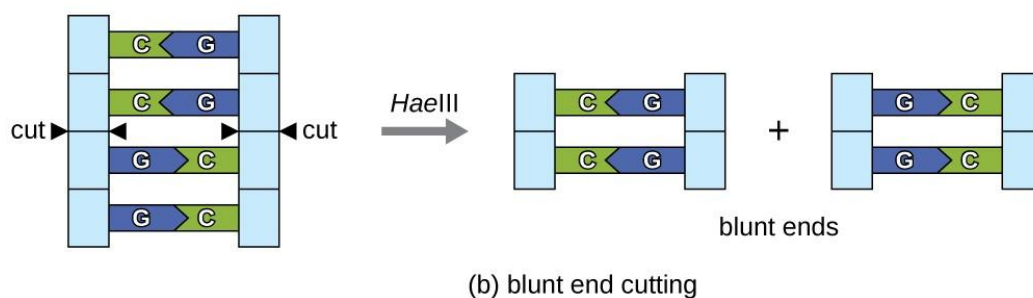
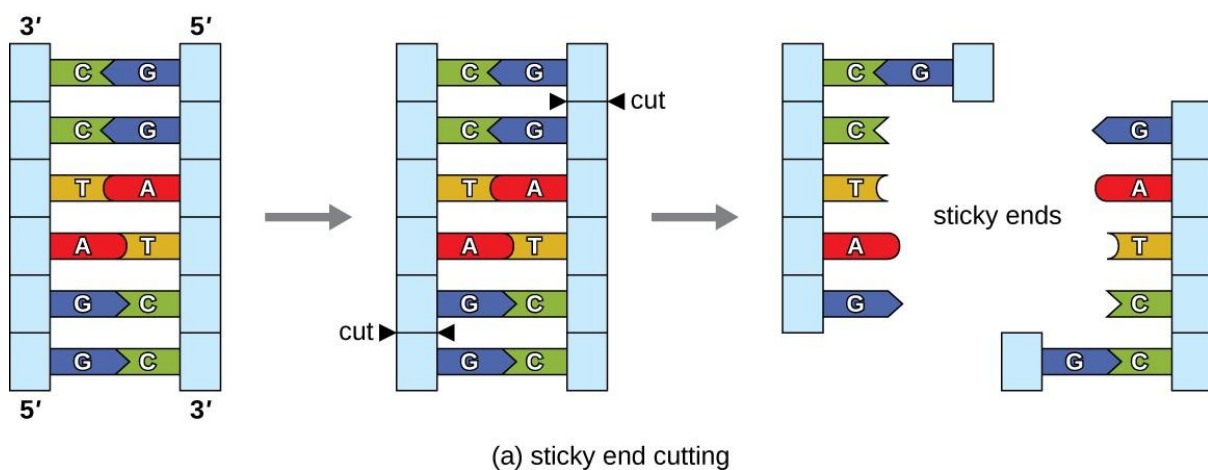
In recombinant DNA technology, DNA molecules are manipulated using naturally occurring enzymes derived mainly from bacteria and viruses

The creation of recombinant DNA molecules is possible due to the use of naturally occurring **restriction endonucleases (restriction enzymes)**, bacterial enzymes produced as a protection mechanism to cut and destroy foreign cytoplasmic DNA that is most commonly a result of bacteriophage infection. Stewart **Linn** and Werner **Arber** discovered restriction enzymes in their 1960s studies of how *E. coli* limits bacteriophage replication on infection.

Each restriction enzyme cuts DNA at a characteristic **recognition site**, a specific, usually palindromic. A restriction enzyme recognizes the DNA palindrome and cuts each backbone at identical positions in the palindrome. Some restriction enzymes cut to produce molecules that have complementary overhangs (**sticky ends**) while others cut without generating such overhangs, instead producing **blunt ends**

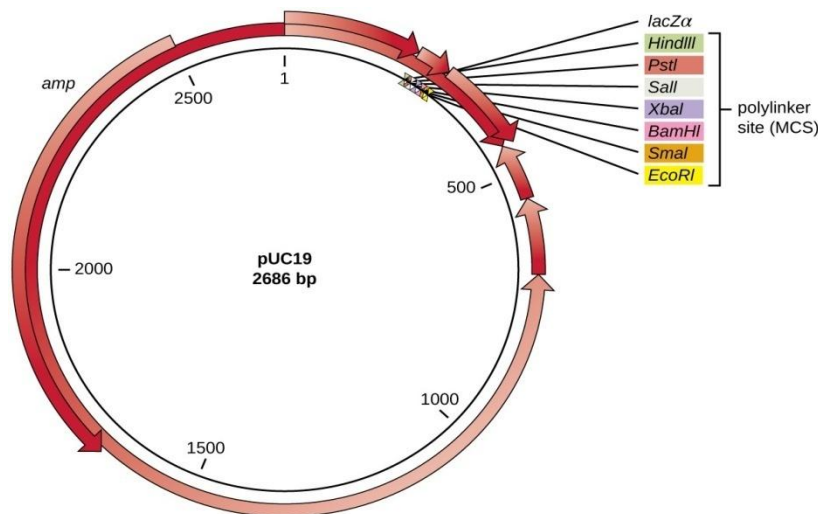
Molecules with complementary sticky ends can easily **anneal**, or form hydrogen bonds between complementary bases, at their sticky ends. The annealing step allows **hybridization** of the single-stranded overhangs. Hybridization refers to the joining together of two complementary single strands of DNA. Blunt ends can also attach together, but less efficiently than sticky ends due to the lack of complementary overhangs facilitating the process.

In either case, **ligation** by **DNA ligase** can then rejoin the two sugar-phosphate backbones of the DNA through covalent bonding, making the molecule a continuous double strand. In 1972, Paul **Berg**, a Stanford biochemist, was the first to produce a recombinant DNA molecule using this technique, combining the SV40 monkey virus with *E. Coli* bacteriophage lambda to create a hybrid



Plasmids

After restriction digestion, genes of interest are commonly inserted into **plasmids**, small pieces of typically circular, double-stranded DNA that replicate independently of the bacterial chromosome. In recombinant DNA technology, plasmids are often used as **vectors**, DNA molecules that carry DNA fragments from one organism to another. Plasmids used as vectors can be genetically engineered by researchers and scientific supply companies to have specialized properties, as illustrated by the commonly used plasmid vector **pUC19**. Some **plasmid vectors** contain genes that confer **antibiotic resistance**;

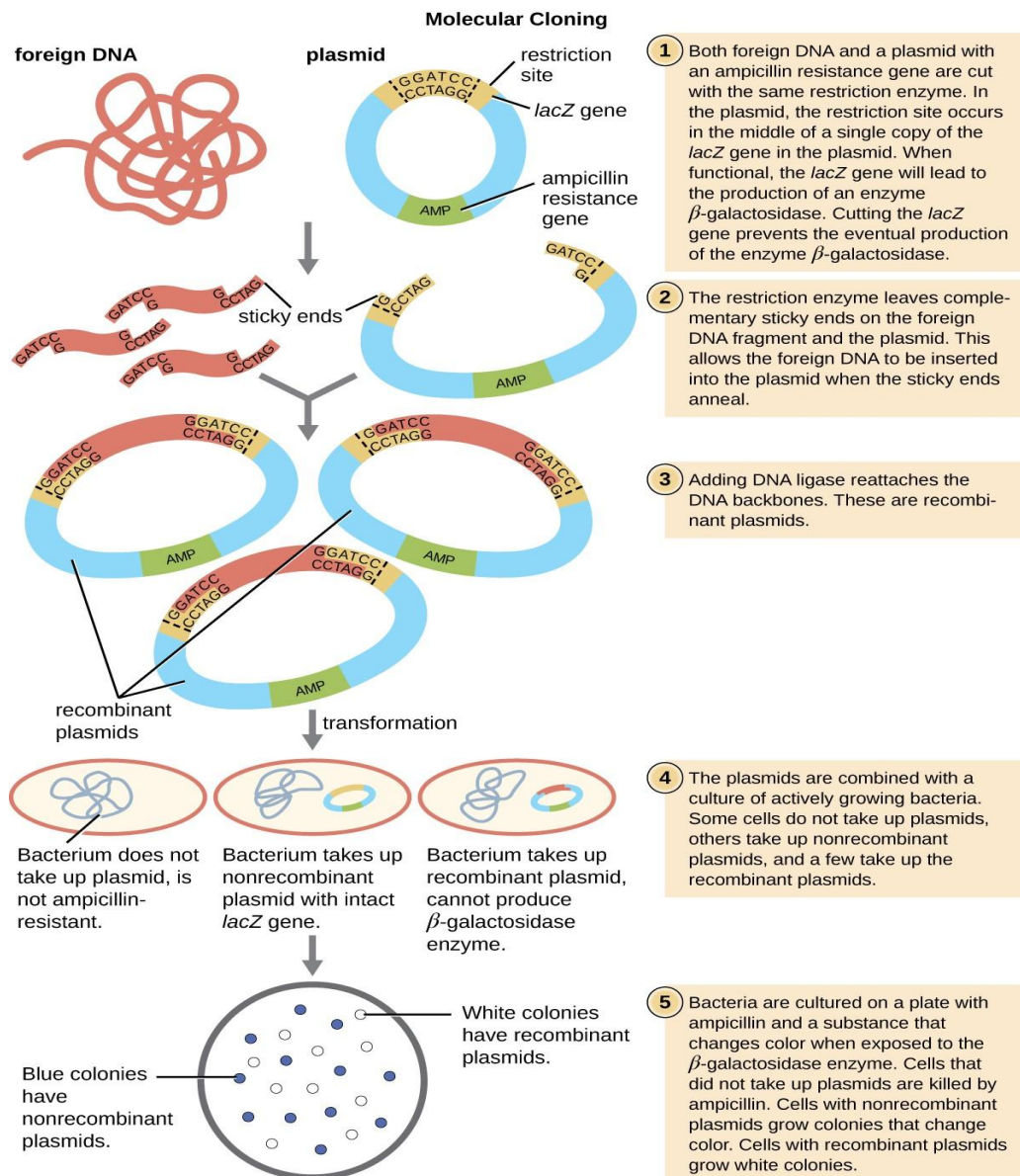


Molecular Cloning using Transformation

The most commonly used mechanism for introducing engineered plasmids into a bacterial cell is **transformation**, a process in which bacteria take up free DNA from their surroundings. In nature, free DNA typically comes from other lysed bacterial cells; in the laboratory, free DNA in the form of recombinant plasmids is introduced to the cell's surroundings.

Some bacteria, such as *Bacillus* spp., are naturally competent, meaning they are able to take up foreign DNA. However, not all bacteria are naturally competent. In most cases, bacteria must be made artificially competent in the laboratory by increasing the permeability of the cell membrane. This can be achieved through chemical treatments that neutralize charges on the cell membrane or by exposing the bacteria to an electric field that creates microscopic pores in the cell membrane. These methods yield chemically competent or electrocompetent bacteria, respectively.

A technique called **blue-white screening** is then used for *lacZ*-encoding plasmid vectors such as pUC19. Blue colonies have no foreign DNA inserted into the polylinker site and white colonies contain plasmids with an insert and can be further screened to characterize the foreign DNA.



Molecular Cloning Using Conjugation

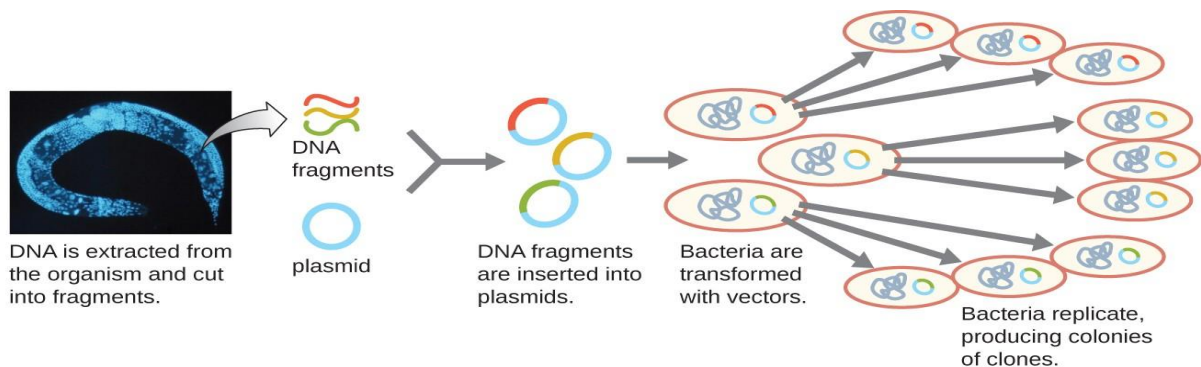
The bacterial process of **conjugation** can also be manipulated for molecular cloning. **F plasmids**, or fertility plasmids, are transferred between bacterial cells through the process of conjugation. Recombinant DNA can be transferred by conjugation when bacterial cells containing a recombinant F plasmid are mixed with compatible bacterial cells lacking the plasmid. F plasmids encode a surface structure called an **F pilus** that facilitates contact between a cell containing an F plasmid and one without an F plasmid. On contact, a cytoplasmic bridge forms between the two cells and the F-plasmid-containing cell replicates its plasmid, transferring a copy of the recombinant F plasmid to the recipient cell. Once it has received the recombinant F plasmid, the recipient cell can produce its own F pilus and facilitate transfer of the recombinant F plasmid to an additional cell. The use of conjugation to transfer recombinant F plasmids to recipient cells is another effective way to introduce recombinant DNA molecules into host cells.

Molecular Cloning Using Transduction

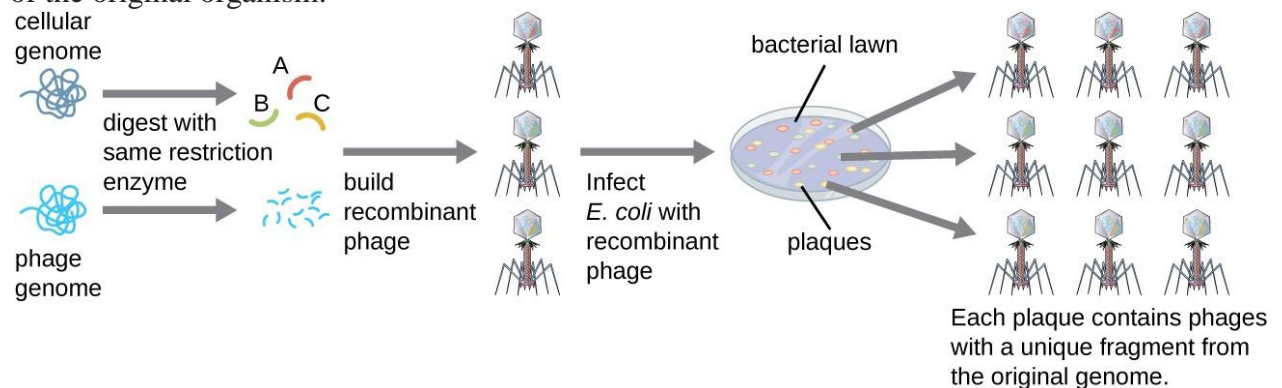
Alternatively, **bacteriophages** can be used to introduce recombinant DNA into host bacterial cells through a manipulation of the **transduction** process. In the laboratory, DNA fragments of interest can be engineered into **phagemids**, which are plasmids that have phage sequences that allow them to be packaged into bacteriophages. Bacterial cells can then be infected with these bacteriophages so that the recombinant phagemids can be introduced into the bacterial cells. Depending on the type of phage, the recombinant DNA may be integrated into the host bacterial genome (lysogeny), or it may exist as a plasmid in the host's cytoplasm.

Creating a Genomic Library

Molecular cloning may also be used to generate a **genomic library**. The library is a complete (or nearly complete) copy of an organism's genome contained as recombinant DNA plasmids engineered into unique clones of bacteria. Having such a library allows a researcher to create large quantities of each fragment by growing the bacterial host for that fragment. These fragments can be used to determine the sequence of the DNA and the function of any genes present.



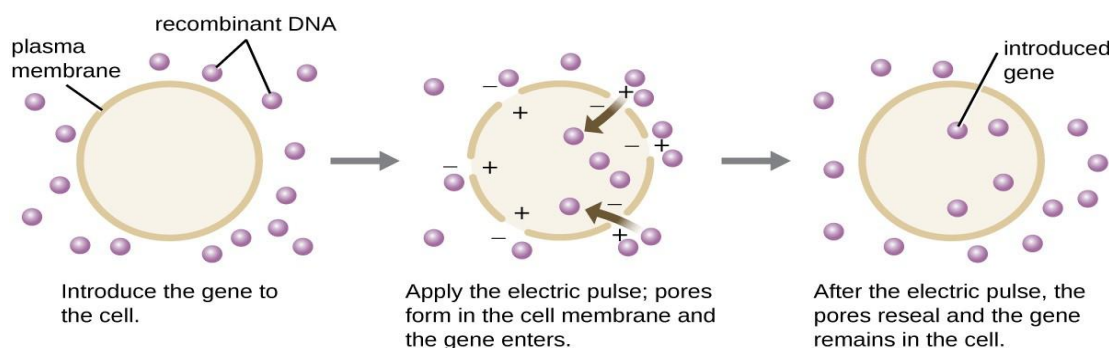
To construct a genomic library using larger fragments of genomic DNA, an *E. coli* bacteriophage, such as **lambda**, can be used as a host. Genomic DNA can be sheared or enzymatically digested and ligated into a pre-digested bacteriophage lambda DNA vector. Then, these recombinant phage DNA molecules can be packaged into phage particles and used to infect *E. coli* host cells on a plate. During infection within each cell, each recombinant phage will make many copies of itself and lyse the *E. coli* lawn, forming a plaque. Thus, each plaque from a phage library represents a unique recombinant phage containing a distinct genomic DNA fragment. Plaques can then be screened further to look for genes of interest. One advantage to producing a library using phages instead of plasmids is that a phage particle holds a much larger insert of foreign DNA compared with a plasmid vector, thus requiring a much smaller number of cultures to fully represent the entire genome of the original organism.



Introducing Recombinant Molecules into Eukaryotic Hosts

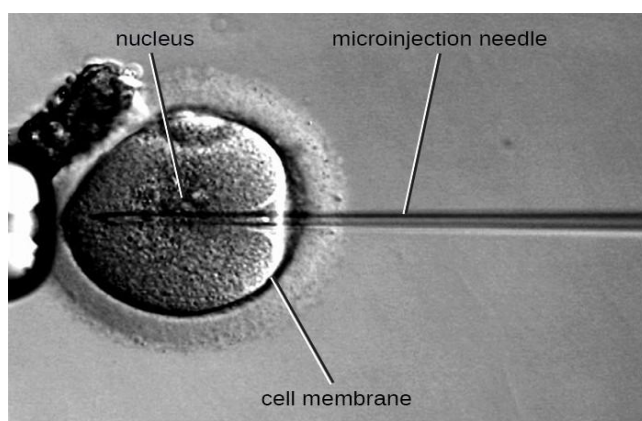
The use of bacterial hosts for genetic engineering laid the foundation for recombinant DNA technology; however, researchers have also had great interest in genetically engineering eukaryotic cells, particularly those of plants and animals. The introduction of recombinant DNA molecules into eukaryotic hosts is called **transfection**. Genetically engineered plants, called **transgenic plants**, are of significant interest for agricultural and pharmaceutical purposes. The first transgenic plant sold commercially was the **Flavr Savr** delayed-ripening tomato, which came to market in 1994. Genetically engineered livestock have also been successfully produced, resulting, for example, in pigs with increased nutritional value and goats that secrete pharmaceutical products in their milk

Electroporation. Compared to bacterial cells, eukaryotic cells tend to be less amenable as hosts for recombinant DNA molecules. Because eukaryotes are typically neither competent to take up foreign DNA nor able to maintain plasmids, transfection of eukaryotic hosts is far more challenging and requires more intrusive techniques for success. One method used for transfecting cells in cell culture is called **electroporation**. A brief electric pulse induces the formation of transient pores in the phospholipid bilayers of cells through which the gene can be introduced. At the same time, the electric pulse generates a short-lived positive charge on one side of the cell's interior and a negative charge on the opposite side; the charge difference draws negatively charged DNA molecules into the cell



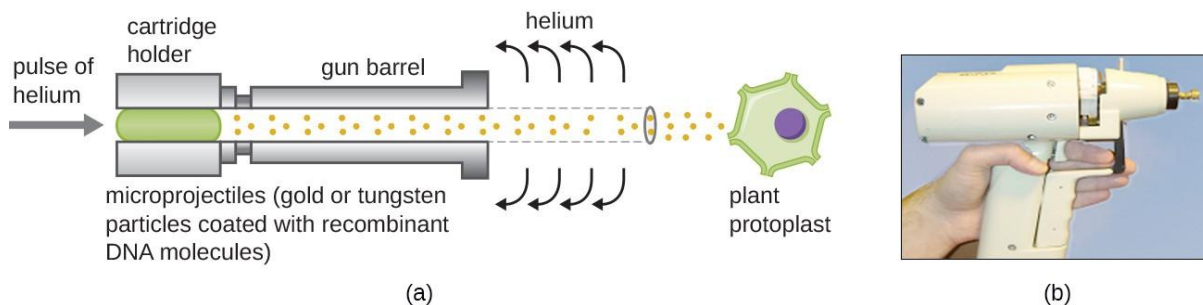
Microinjection

An alternative method of transfection is called **microinjection**. Because eukaryotic cells are typically larger than those of prokaryotes, DNA fragments can sometimes be directly injected into the cytoplasm using a glass micropipette,



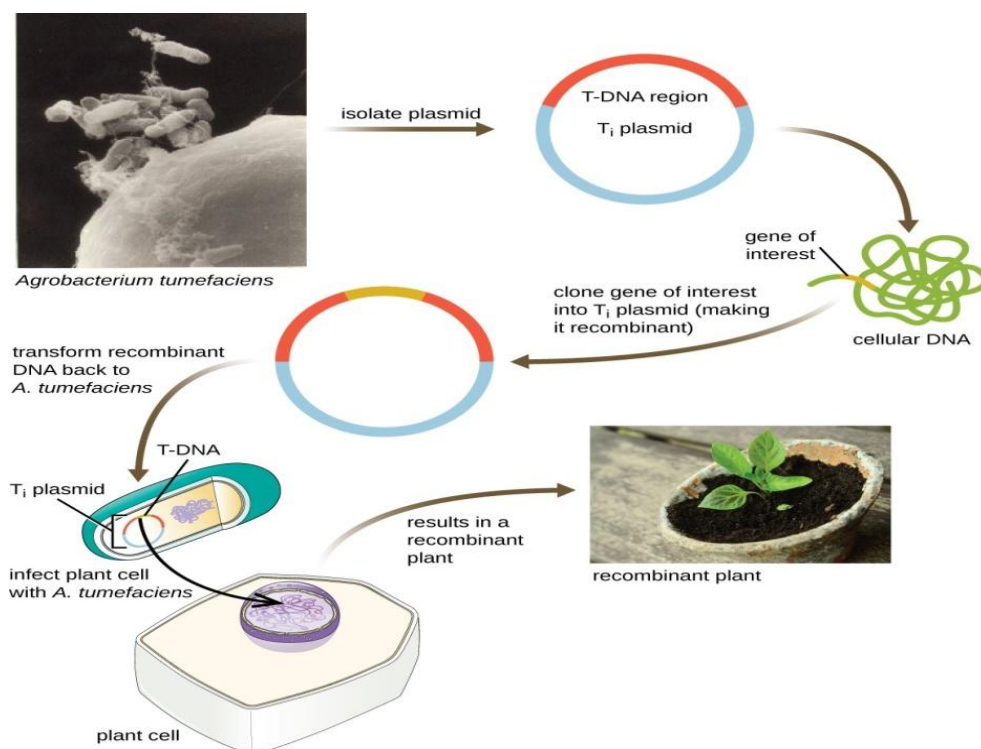
Gene Guns

Transfecting plant cells can be even more difficult than animal cells because of their thick cell walls. One approach involves treating plant cells with enzymes to remove their cell walls, producing protoplasts. Then, a **gene gun** is used to shoot gold or tungsten particles coated with recombinant DNA molecules into the plant protoplasts at high speeds. Recipient protoplast cells can then recover and be used to generate new transgenic plants



Shuttle Vectors

Another method of transfecting plants involves **shuttle vectors**, plasmids that can move between bacterial and eukaryotic cells. The **tumor-inducing (T_i) plasmids** originating from the bacterium *Agrobacterium tumefaciens* are commonly used as shuttle vectors for incorporating genes into plants. In nature, the T_i plasmids of *A. tumefaciens* cause plants to develop tumors when they are transferred from bacterial cells to plant cells. Researchers have been able to manipulate these naturally occurring plasmids to remove their tumor-causing genes and insert desirable DNA fragments. The resulting recombinant T_i plasmids can be transferred into the plant genome through the natural transfer of T_i plasmids from the bacterium to the plant host. Once inside the plant host cell, the gene of interest recombines into the plant cell's genome.



Viral Vectors

Viral vectors can also be used to transfect eukaryotic cells. In fact, this method is often used in **gene therapy** (see [Gene Therapy](#)) to introduce healthy genes into human patients suffering from diseases that result from genetic mutations. Viral genes can be deleted and replaced with the gene to be delivered to the patient;^[4] the virus then infects the host cell and delivers the foreign DNA into the genome of the targeted cell. Adenoviruses are often used for this purpose because they can be grown to high titer and can infect both nondividing and dividing host cells. However, use of **viral vectors** for gene therapy can pose some risks for patients, as discussed in [Gene Therapy](#).

- **Biotechnology** is the science of utilizing living systems to benefit humankind. In recent years, the ability to directly alter an organism's genome through **genetic engineering** has been made possible due to advances in **recombinant DNA technology**, which allows researchers to create **recombinant DNA molecules** with new combinations of genetic material.
- **Molecular cloning** involves methods used to construct recombinant DNA and facilitate their replication in host organisms. These methods include the use of **restriction enzymes** (to cut both foreign DNA and **plasmid vectors**), **ligation** (to paste fragments of DNA together), and the introduction of recombinant DNA into a host organism (often bacteria).
- **Blue-white screening** allows selection of bacterial transformants that contain recombinant plasmids using the phenotype of a **reporter gene** that is disabled by insertion of the DNA fragment.
- **Genomic libraries** can be made by cloning genomic fragments from one organism into plasmid vectors or into bacteriophage.
- **cDNA libraries** can be generated to represent the mRNA molecules expressed in a cell at a given point.
- **Transfection** of eukaryotic hosts can be achieved through various methods using **electroporation**, **gene guns**, **microinjection**, **shuttle vectors**, and **viral vectors**.