

A Basic Primer on Biotechnology

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Biotechnology and its use to modify the genetic makeup of living organisms has become a topic of heated discussion in recent years. Confusion is plentiful on the topic of biotechnology and genetically modified organisms (GMOs). The purpose of this publication is to discuss genetic/biochemical processes at a basic level and how biotechnology is used to modify the genetic makeup of an organism.

Genome

The complete set of genetic instructions for a living organism is contained in its genetic code, referred to as its genome. The genome for each organism differs by the number and size of chromosomes and the number of genes each contains. Each chromosome is composed of a single strand of deoxyribonucleic acid (DNA) and specialized protein molecules (Figure 1a and Figure 1b). Coding regions called genes are along the DNA strand of each chromosome. Only specific regions of each chromosome code for genes. Alternate forms of genes in each organism account for the differences between individuals. Each DNA strand is composed of similar repeating units called nucleotides (Figure 1c). Four different nucleotide bases are present in DNA. They are adenine (A), thymine (T), cytosine (C), and guanine (G). The specific order of these bases in a gene coding region on the DNA strand specify exact genetic instructions.

Two DNA strands are held together by bonds between the bases; these constitute base pairs. Often the size of a genome is referred to by its number of base pairs. Each time a cell divides, the full genome is replicated and each daughter cell receives an exact copy of the genetic code (Figure 1d). Each strand of DNA directs the synthesis of a complementary strand with free nucleotides matching up with their new complementary bases on each of the strands. Strict base pairing is adhered to; A will only pair with T,

and C will only pair with G. Each daughter cell receives one old and one new DNA strand (Figure 1e).

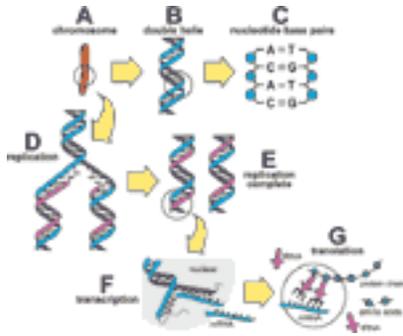


Figure 1. The basic structure and function of chromosomes and genes.
(click on image for a 20KB color illustration)

Genes

The genes on each DNA strand contain the basic physical and functional units of heredity. A gene is a specific sequence of nucleotide bases, whose sequences carry the information required for constructing proteins. In turn, proteins regulate the expression of the genes and provide structural components and enzymes for biochemical reactions necessary for all living organisms.

The protein-coding instructions from genes are transmitted indirectly through messenger ribonucleic acid (mRNA), a transient intermediary molecule similar to a single strand of DNA. For the information within a gene to be expressed, a complementary RNA strand is produced (by a process called transcription) from the DNA template in the nucleus (Figure 1f). This mRNA is moved from the nucleus to the cellular cytoplasm, where it serves as the template for protein synthesis. The cell's protein-synthesizing machinery then translates the genetic code, or codons, into a string of amino acids that will constitute the protein molecule (by a process called translation) encoded by the gene (Figure 1g). Following modification, the resulting protein can begin its function either as an enzyme, structural or regulatory protein.

Proteins are large, complex molecules made up of long chains of amino acid subunits. There are 20 different amino acids. Within a gene, each specific sequence of three DNA bases (codons) directs the cell's protein-synthesizing machinery to add a specific amino acid. For example, the base sequence ATG codes for the amino acid methionine (any biochemistry text will have a complete list of amino acids and their corresponding codons). The genetic code is thus a series of codons that specify which amino acids are required to make the specific protein a gene codes for. The genetic code is the same for all living organisms.

Not all genes are expressed in all tissues. For example, the tassel and developing ears on a corn plant (*Zea mays*) produce pollen and embryos that will develop into seed. The differences between these two plant parts are ultimately controlled by gene expression. The differential expression of genes is

controlled by its promoter. The expression of a few genes in plants are controlled by environmental factors such as sunlight, temperature, and day length. These three factors are important in triggering flowering in many plant species.

Uses of Biotechnology

Biotechnology includes a vast array of tools used in research and modification of biological systems. These include: **genetic mapping**, the process of identifying the location of a gene on a chromosome and elucidating the gene sequence; **molecular based disease diagnosis**, identifying specific alleles (alternate forms of a gene) of a gene which cause genetic diseases; **gene therapy**, replacing an absent or defective gene with a working one enabling normal body function; **forensic science**, solving crimes and identifying human remains not previously possible; and **genetic transformation**, movement of a gene or group of genes from one organism to another.

Genetic Transformation

Genetic transformation is the area of biotechnology that has created the greatest amount of stir and which will be the focus from this point on. Organisms with genetic material from another organism are often referred to as genetically modified organisms or GMOs. Since all crop and domesticated animal species have been genetically modified since the dawn of time, technically they are also GMOs. When referring to organisms with a gene from another species, transgenic is a more accurate description.

Many of the processes of biotechnology have been used for many years. Insulin from pigs and cows was historically used to treat diabetes and was beneficial to a many. However, there was not a consistent supply and some individuals developed adverse reactions to this type of insulin because their bodies recognized it as foreign and mounted an immune response. Human insulin produced through cloning and inserting human genes in bacteria resulted in insulin that did not cause an immune response. This was the first pharmaceutical produced through biotechnology and it has insured a consistent reliable source of human insulin.

Before a gene is transferred to another organism it must be identified, isolated and cloned. In the laboratory, the mRNA molecule from a gene being expressed can be isolated and used as a template to synthesize a complementary DNA (cDNA) strand. This isolated cDNA strand can then be cloned (duplicated) for transformation into another species. The cDNA strand can be used to locate the corresponding gene on a chromosome, or map it.

Transformation is typically accomplished by using either *Agrobacterium tumefaciens* or particle acceleration and the gene gun (Figure 2). *Agrobacterium tumefaciens* is a bacteria that occurs in nature. It contains a small circular piece of DNA called a *Ti* plasmid (*Ti* for tumor inducing). When this bacterium infects certain woody plant species, the *Ti* plasmid enters cells of the host plant. Certain regions of the *Ti* plasmid insert themselves into the host cell's genome. This insertion occurs in a region of the DNA strand with a specific sequence. The host cell then expresses the gene from the bacteria, which induces massive cell growth and the resultant plant tumor the bacteria is named for (Figure 3). Biotechnology utilizes this natural transformation process by removing the bacterial genes from the region transferred to the host genome and substituting genes of interest (Figure 2a). *Agrobacterium* use for transformation is limited because it will only infect certain dicotyledonous species.

The other transformation process involves coating gold particles with genes of interest. The gold particles are shot into single cells of the plant of interest with the gene gun. This is commonly referred to as particle acceleration. In a process not fully understood, the transgene(s) are incorporated into a DNA strand of the host genome (Figure 2b). This process is inefficient but does not have the host species limitation of *Agrobacterium*.

Both processes require the use of plant tissue culture. Individual cells of the plant to be transformed are cultured. These are then subjected to the transformation process. Non-transformed cells must be eliminated. This is done with selectable marker genes. In the case of the Roundup Ready gene, Roundup (glyphosate) is used directly as the selectable marker, since Roundup will kill non-transformed cells (Figure 2c). When another trait of interest is being transformed in the crop, a selectable marker like antibiotic or herbicide resistance is used. The cells in culture are treated with the herbicide or an antibiotic. Only those cells that were transformed with the two genes will survive. Whole plants are then regenerated from the single cells that survive.

Following transformation and plant regeneration, the transgenic plants must be tested in the field to ensure that the transgene functions properly. Not all transgenic plants will express the trait or gene product properly. Once a transgenic plant that expresses the trait has been identified and is stable, then the trait can be bred using conventional plant breeding methods into cultivars with adaptation to the environmental conditions where the crop is produced.

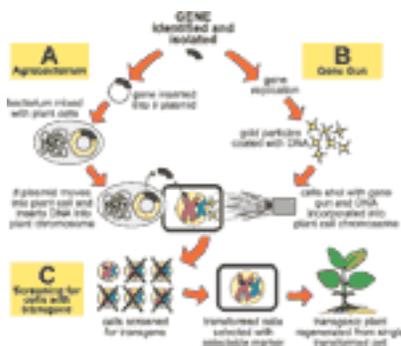


Figure 2. The basic process of plant transformation with *Agrobacterium* and the gene gun. (click on image for a 26KB color illustration)



Figure 3. Crown gall resulting when bacterial DNA is naturally transformed into the tree. (Used with permission from University of California Statewide IPM Project, J.K. Clark, photographer) (click on image for a 37KB color photograph)

Examples of Transgenic Crops

The two most common and well known transgenic events are for resistance to the herbicide glyphosate (Roundup) and *Bacillus thuringiensis* (*Bt*). Both of these traits are for pest management. The Flavr Savr tomato is an example of genetic modification, using recombinant DNA technology, designed to preserve the freshness of tomatoes. In the Flavr Savr tomato, resident DNA was modified and reinserted into the tomato. The failure of Flavr Savr tomato has been attributed to the poor taste of the cultivar.

Roundup, one of the most widely used and safest herbicides to humans and the environment, functions by inhibiting activity of the enzyme 5-enolpyruvylshiki-mate-3-phosphate synthase (EPSPS). The EPSPS enzyme is critical in the metabolic pathway leading to development of the three aromatic amino acids phenylalanine, tyrosine, and tryptophan. All plants synthesize the amino acids needed for plant growth and function. A blocked metabolic pathway critical for synthesis of the amino acids eliminates the plant's ability to make required proteins and enzymes and results in plant death.

A bacteria was discovered that contained an alternate form of the EPSPS enzyme, called CP4-EPSPS, that is not inhibited by glyphosate, the active ingredient in Roundup. The difference between the two genes that code for the enzyme are slight. A single point mutation in the gene switched the nucleotide guanine for cytosine, which causes the amino acid alanine to be substituted for glycine and prevents glyphosate from binding the enzyme, allowing the Shikimate pathway to function normally (Figure 4). The gene from the bacteria that codes for the CP4 form of EPSPS was modified by adding a promoter that is recognized by plants. This modified gene was inserted into soybean to create the Roundup Ready soybean. Since the soybean contains the CP4-EPSPS enzyme, which is not inhibited by glyphosate, the modified "Roundup Ready" soybean will not be killed by Roundup.

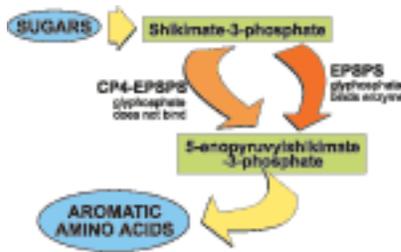


Figure 4. In the shikimate pathway glyphosate binds the EPSPS enzyme. Plants with the transgene that codes for CP4-EPSPS function normally because glyphosate does not bind the CP4-EPSPS form.

The *Bt* trait in corn, cotton, and potato is another very popular transgenic trait. The *Bt* stands for *Bacillus thuringiensis*, a bacterium. Research conducted years ago found that proteins from this bacteria bind to the gut of insect larva and kill them. These proteins have been isolated and used as insecticides for many years. What makes *Bt* crops unique is that the genes that code for these proteins were isolated from the bacteria, modified with promoters that would be recognized by plants and inserted into the crop species. The plant then makes the particular *Bt* protein coded for by the gene inserted into that crop. A corn hybrid with a *Bt* gene encodes crystalline proteins from the bacteria that are responsible for larvae toxicity. When eaten by the European corn borer, these crystalline, or Cry proteins, bind to the insects' midgut causing a water imbalance in the cells. The cells burst killing the corn borer. *Bt* cotton where the target pest is the boll weevil functions similarly. The *Bt* trait is unique in that multiple *Bt* genes are used to target different insect pests in different crops.

As discussed earlier, not all genes are expressed in all tissues. If a promoter is used with the *Bt* gene inserted into a crop that is expressed in all tissue, then the trait is effective in all plant parts. For most *Bt* transgenic events, the CaMV35S promoter is used, which expresses in all tissue, including the pollen (Figure 5a). When pollen from *Bt* corn drifts to other plants, it could result in the death of non-target insects.

An alternate promoter sequence to the CaMV35S is the phosphoenolpyruvate (PEP) carboxylase promoter from a plant gene encoding a photosynthetic enzyme. The result is, the *Bt* transgene with this promoter will produce the protein only in cells that are actively making photosynthetic proteins. Hence the root, tassel, or ear tissue in *Bt* corn are not expressing the *Bt* trait (Figure 5b). The down side of this is that insect pests that attack these tissues are not controlled; furthermore, expression also begins to slow and eventually stops toward the end of the season when the plant is completing its life cycle and photosynthesis is reduced.

Transgenic crops, and biotechnology in general, have tremendous potential to benefit producers, processors and consumers. The examples used here are mostly limited to those that aid the producer who grows the crop. As advances in biotechnology are made it is important to learn and understand the technology to avoid confusion and misinformation that could prevent the potential benefits of the technology from being realized.

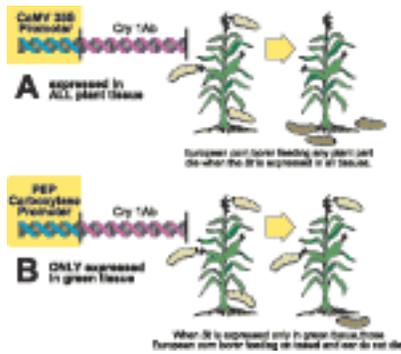


Figure 5. A single *Bt* gene being expressed differentially in corn based on the promoter.

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