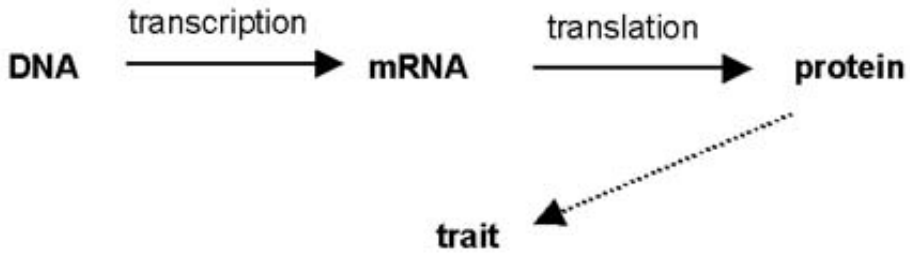
How Do You Make A Transgenic Plant?

How to Make Transgenic Plants:
An animation from the University of Nebraska at Lincoln

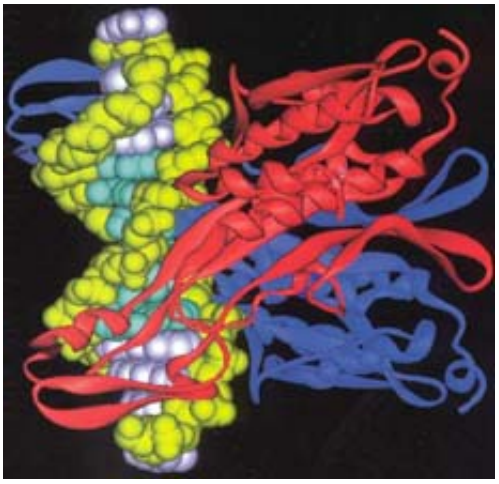
How Do We Develop Transgenic Plants:
An animation from the Saskatchewan Agricultural Biotechnology Information Centre

Introduction to DNA

The underlying reason that transgenic plants can be constructed is the universal presence of **DNA** (deoxyribonucleic acid) in the cells of all living organisms. This molecule stores the organism's genetic information and orchestrates the metabolic processes of life. Genetic information is specified by the sequence of four chemical bases (adenine, cytosine, guanine, and thymine) along the length of the DNA molecule. **Genes** are discrete segments of DNA that encode the information necessary for assembly of a specific protein. The proteins then function as enzymes to catalyze biochemical reactions, or as structural or storage units of a cell, to contribute to expression of a plant trait. The general sequence of events by which the information encoded in DNA is expressed in the form of proteins via an mRNA intermediary is shown in the diagram below.



The transcription and translation processes are controlled by a complex set of regulatory mechanisms, so that a particular protein is produced only when and where it is needed. For more information on molecular genetics, consult any recent genetics text or the web site Access Excellence, Graphics Gallery http://www.accessexcellence.org/ . Even species that are very different have similar mechanisms for converting the information in DNA into proteins; thus, a DNA segment from bacteria can be interpreted and translated into a functional protein when inserted into a plant.



Among the most important tools in the genetic engineer's tool kit are enzymes that perform specific functions on DNA. The image at left (Voet, Donald 1995 Biochemistry) shows the structure of DNA as a double helix with the phosphate backbone in yellow-green and the bases in white or teal green. The blue and red figures represent the 3-D structure of a **restriction enzyme** (EcoR1) which recognizes and cuts the DNA at a specific region of the DNA. Other enzymes known as **ligases** join the ends of two DNA fragments. These and other enzymes enable the manipulation and amplification of DNA, essential components in joining the DNA of two unrelated organisms.

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Locating Genes for Plant Traits

Identifying and locating genes for agriculturally important traits is currently the most limiting step in the transgenic process. We still know relatively little about the specific genes required to enhance yield potential, improve stress tolerance, modify chemical properties of the harvested product, or otherwise affect plant characters. Usually, identifying a single gene involved with a trait is not sufficient; scientists must understand how the gene is regulated, what other effects it might have on the plant, and how it interacts with other genes active in the same biochemical pathway. Public and private research programs are investing heavily into new technologies to rapidly sequence and determine functions of genes of the most important crop species. These efforts should result in identification of a large number of genes potentially useful for producing transgenic varieties.

The techniques for locating and sequencing stretches of DNA that control specific traits are beyond the scope of this web site. The interested reader is referred to Klug and Cummings, 1998, Lewin, 1999; Wong, 1997, or other recent genetics texts.

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Designing Genes for Insertion

Once a gene has been isolated and cloned (amplified in a bacterial vector), it must undergo several modifications before it can be effectively inserted into a plant.

Simplified representation of a constructed transgene, containing necessary components for successful integration and expression.

1. A **promoter sequence** must be added for the gene to be correctly expressed (i.e., translated into a protein product). The promoter is the on/off switch that controls when and where in the plant the gene will be expressed. To date, most promoters in transgenic crop varieties have been "constitutive", i.e., causing gene expression throughout the life cycle of the plant in most tissues. The most commonly used constitutive promoter is CaMV35S, from the

cauliflower mosaic virus, which generally results in a high degree of expression in plants. Other promoters are more specific and respond to cues in the plant's internal or external environment. An example of a light-inducible promoter is the promoter from the cab gene, encoding the major chlorophyll a/b binding protein.

2. Sometimes, the **cloned gene is modified** to achieve greater expression in a plant. For example, the Bt gene for insect resistance is of bacterial origin and has a higher percentage of A-T nucleotide pairs compared to plants, which prefer G-C nucleotide pairs. In a clever modification, researchers substituted A-T nucleotides with G-C nucleotides in the Bt gene without significantly changing the amino acid sequence. The result was enhanced production of the gene product in plant cells.

3. The **termination sequence** signals to the cellular machinery that the end of the gene sequence has been reached.

4. A **selectable marker gene** is added to the gene "construct" in order to identify plant cells or tissues that have successfully integrated the transgene. This is necessary because achieving incorporation and expression of transgenes in plant cells is a rare event, occurring in just a few percent of the targeted tissues or cells. Selectable marker genes encode proteins that provide resistance to agents that are normally toxic to plants, such as antibiotics or herbicides. As explained below, only plant cells that have integrated the selectable marker gene will survive when grown on a medium containing the appropriate antibiotic or herbicide. As for other inserted genes, marker genes also require promoter and termination sequences for proper function.

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Transforming Plants

Transformation is the heritable change in a cell or organism brought about by the uptake and establishment of introduced DNA. There are two main methods of transforming plant cells and tissues:

1. The **"Gene Gun" method** (also known as microprojectile bombardment or biolistics). This technique, which is shown and explained in the animated demo section of this web site, has been especially useful in transforming monocot species like corn and rice.
2. The **Agrobacterium method**, which is described below. Transformation via Agrobacterium has been successfully practiced in dicots (broadleaf plants like soybeans and tomatoes) for many years, but only recently has it been effective in monocots (grasses and their relatives). In general, the Agrobacterium method is considered preferable to the gene gun, because of the greater frequency of single-site insertions of the foreign DNA, making it easier to monitor.

Agrobacterium Method of Plant Transformation

Agrobacterium tumefaciens is a remarkable species of soil-dwelling bacteria that has the ability to infect plant cells with a piece of its DNA. When the bacterial DNA is integrated into a plant chromosome, it effectively hijacks the plant's cellular machinery and uses it to ensure the proliferation of the bacterial population. Many gardeners and orchard owners are unfortunately familiar with A. *tumefaciens*, because it causes crown gall diseases in many ornamental and fruit plants.

Crown gall of raspberry caused by *Agrobacterium tumefaciens*.

Source: Ohio State University

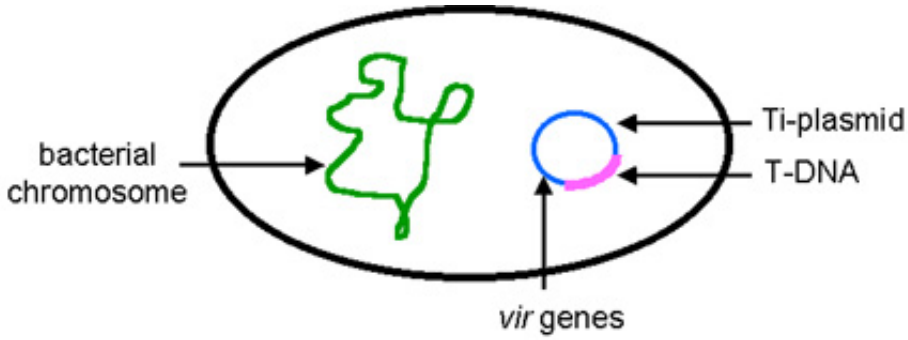

Diagram of *Agrobacterium tumefaciens* cell

The DNA in an A. *tumefaciens* cell is contained in the bacterial chromosome as well as in another structure known as a Ti (tumor-inducing) plasmid. The Ti plasmid contains

- a stretch of DNA termed T-DNA (~20 kb long) that is transferred to the plant cell in the infection process.
- a series of *vir* (virulence) genes that direct the infection process.

A *tumefaciens* can only infect a plant through wounds. When a plant root or stem is wounded it gives off certain chemical signals. In response to those signals, the *vir* genes of A. *tumefaciens* become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant's chromosome. Different *vir* genes

- Copy the T-DNA.
- Attach a product to the copied T-DNA strand to act as a leader.
- Add proteins along the length of the T-DNA, possibly as a protective mechanism.
- Open a channel in the bacterial cell membrane, through which the T-DNA passes.

The T-DNA then enters the plant cell through the wound. It is not clear how the bacterial DNA moves from the cytoplasm to the nucleus of the plant cell, nor how the T-DNA becomes integrated into the plant chromosome. Remember that most of the time plant DNA does not exist as an exposed strand, but is wrapped with histone proteins and is in a supercoiled state. One speculation is that the T-DNA waits until the plant DNA is being replicated or transcribed, then inserts itself into the exposed plant DNA (Galun and Breiman, 1997).

To harness A. *tumefaciens* as a transgene vector, scientists have removed the tumor-inducing section of T-DNA, while retaining the T-DNA border regions and the vir genes. The transgene is inserted between the T-DNA border regions, where it is transferred to the plant cell and becomes integrated into the plant's chromosomes

(Wong, 1997).

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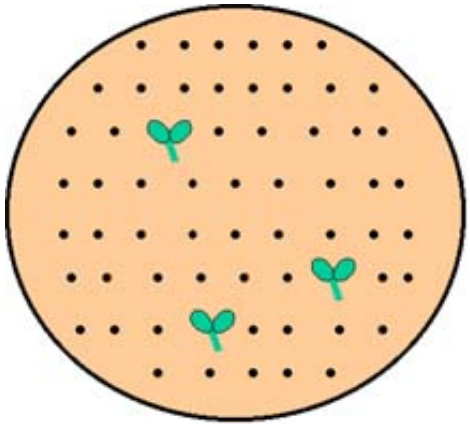
Selection and Regeneration

Selection of successfully transformed tissues. Following the gene insertion process, plant tissues are transferred to a selective medium containing an antibiotic or herbicide, depending on which selectable marker was used. Only plants expressing the selectable marker gene will survive, as shown in the figure, and it is assumed that these plants will also possess the transgene of interest. Thus, subsequent steps in the process will only use these surviving plants.



When grown on selective media, only plant tissues that have successfully integrated the transgene construct will survive.

Regeneration of whole plants. To obtain whole plants from transgenic tissues such as immature embryos, they are grown under controlled environmental conditions in a series of media containing nutrients and hormones, a process known as tissue culture. Once whole plants are generated and produce seed, evaluation of the progeny begins. This regeneration step has been a stumbling block in producing transgenic plants in many species, but specific varieties of most crops can now be transformed and regenerated.

Tissue culture of transgenic plants in a controlled environmental chamber. Source: USDA

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Future Developments in Transgenic Technology

New techniques for producing transgenic plants will improve the efficiency of the process and will help resolve some of the environmental and health concerns. Among the expected changes are the following:

- More efficient transformation, that is, a higher percentage of plant cells will successfully incorporate the transgene.
- Better marker genes to replace the use of antibiotic resistance genes.
- Better control of gene expression through more specific promoters, so that the inserted gene will be active only when and where needed.
- Transfer of multi-gene DNA fragments to modify more complex traits.

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Plant Breeding and Testing

Intrinsic to the production of transgenic plants is an extensive evaluation process to verify whether the inserted gene has been stably incorporated without detrimental effects to other plant functions, product quality, or the intended agroecosystem. Initial evaluation includes attention to:

- Activity of the introduced gene
- Stable inheritance of the gene
- Unintended effects on plant growth, yield, and quality

If a plant passes these tests, most likely it will not be used directly for crop production, but will be crossed with improved varieties of the crop. This is because only a few varieties of a given crop can be efficiently transformed, and these generally do not possess all the producer and consumer qualities required of modern cultivars. The initial cross to the improved variety must be followed by several cycles of repeated crosses to the improved parent, a process known as backcrossing. The goal is to recover as much of the improved parent's genome as possible, with the addition of the transgene from the transformed parent.

The next step in the process is multi-location and multi-year evaluation trials in greenhouse and field environments to test the effects of the transgene and overall performance. This phase also includes evaluation of environmental effects and food safety. For more information on these aspects, please proceed to the Evaluation & Regulation portion of this web site.

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