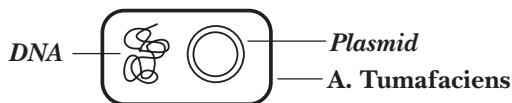


Making Genetically Engineered Plants

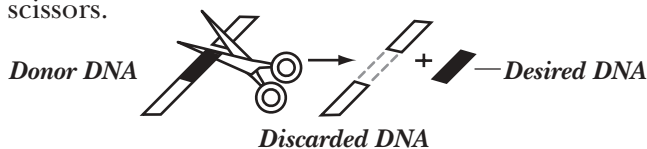
Genetic engineering is a technique that is based on work done by several scientists who won the Nobel Prize. It sounds like science fiction, but it is used widely today in the biotechnology industry and at university research laboratories. The actual technique is pretty well defined. But the claims made about it are often not accurate. Here is a description of how it is done and one example of how it has been applied in plants. Answers to some common questions about this technique follow these two descriptions. Definitions of terms used are on the last page of this publication.

HOW IS GENETIC ENGINEERING DONE IN PLANTS?

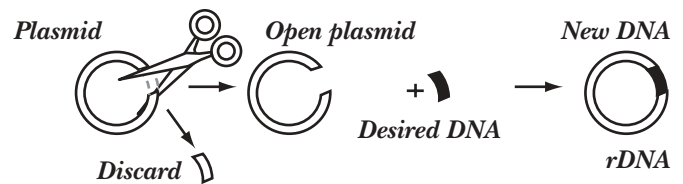
Scientists usually use a “natural” genetic engineer to help them do this. The natural genetic engineer is a common soil bacterium called *Agrobacterium tumefaciens* or *A. tumefaciens*. This bacterium contains a small circle of free-floating DNA called a plasmid. The bacterium normally uses this plasmid to transform certain plants causing crown gall disease.



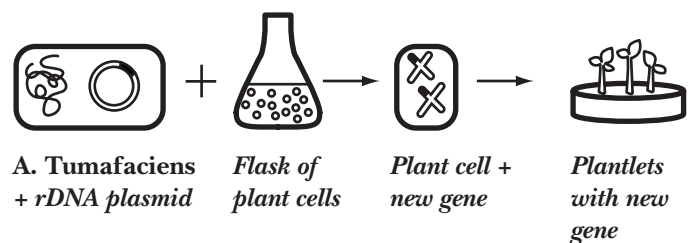
Scientists will first identify the gene that controls the desired characteristic or trait they want to transfer to the target plant. They can remove the gene or piece of DNA that has this trait from the donor organism using special enzymes that act like scissors.



They can also take the plasmid out of *A. tumefaciens* and snip out the DNA from the plasmid that causes crown gall disease, making an open circle of DNA. Then they mix the “open” plasmid with the gene taken from the donor organism. By using special enzymes that paste DNA together, they can produce a plasmid that contains the desired gene. This plasmid is called recombinant DNA or rDNA.



Scientists then put the recombinant plasmid back into *A. tumefaciens*. These bacteria containing the altered plasmid are mixed with cells or tissue from the target plant. Some of the plant cells accept the desired gene from the plasmid and insert it into their own DNA. When these plant cells are grown in tissue culture to small plants, they can be tested to see if they have incorporated the new gene. Those that have are called transformed plants and are tested further.



WHAT IS A PRACTICAL EXAMPLE OF THIS TECHNIQUE?

Summer squash like zucchini are attacked by a number of viruses. These viruses kill the plants and reduce the yield of squash from a farmer's field. Viruses are very small organisms that are composed of specific DNA enclosed in a capsule or coat of protein. Scientists have learned that if a plant contains the gene for the protein making the coat (coat protein) of the virus, it is resistant to the virus. Using this information, scientists have produced squash plants that are resistant to specific viruses.

The scientists snip out the gene for the virus coat protein from the viral DNA. As described above, they create open plasmids from *A. tumefaciens*. When they mix these open plasmids with the viral coat protein genes and specific enzymes they produce recombinant plasmids that contain the viral coat protein gene. The recombinant plasmids are reintroduced into *A. tumefaciens* and produce bacteria containing the rDNA plasmid.

The *A. tumefaciens* containing the rDNA plasmid are mixed with the target squash cells in tissue culture. Some of the squash cells take up the virus coat protein gene from *A. tumefaciens* and integrate it into their own chromosomal DNA. These cells grow into plants and are tested to see if they are resistant to the virus. Virus-resistant plants are further tested.



Traditional variety of squash.



Squash genetically engineered to be virus resistant.

ARE THERE OTHER WAYS TO DO GENETIC ENGINEERING?

Yes. Scientists also use a "shotgun method." In this case, the scientist isolates the gene or genes of interest. This DNA is mixed with and sticks to microscopic gold or tungsten pellets. These are used like very small shotgun pellets. The DNA-coated pellets are placed on a support that is in front of an array of target plant cells. A blast of helium gas is aimed at the pellets so they are shot at the target plant cells. Some of the pellets hit and enter the cells. Some of these cells hit by the DNA pellet incorporate the foreign DNA into their own DNA. These cells are grown into plants and tested. This method is used in plants like wheat, rice, and corn where *Agrobacterium* is not suitable to use.

COMPANIES PROMOTING GENETIC ENGINEERING CLAIM THAT IT IS MORE EXACT AND PRECISE THAN TRADITIONAL CROSS BREEDING. IS THIS TRUE?

Yes and no. Traditional cross breeding involves the transfer and sorting of thousands of genes from two parent plants into offspring. The plant breeder then has to sort through all the offspring to find the ones with the characteristics or traits of interest. It takes several generations and a number of years before an offspring is identified and developed for market that has the specific trait desired.

Genetic engineering is more precise in that scientists identify the specific gene or genes they want to move from the donor organism. This reduces the number of genes that are transferred to the recipient organism to only those for the characteristic of interest.

Currently, scientists cannot control where the donor gene is inserted into the DNA of the recipient or the number of copies of the rDNA inserted. The site where the donor DNA is inserted and the number of copies of the rDNA inserted are likely to control how the desired trait is expressed in the plant. So the plant breeder still has to do field testing to find the best offspring. Therefore, this part of the process is not yet as precise as some would claim.

For example, scientists in Europe recently found that Roundup Ready soybeans contain more than just one complete copy of the transferred

gene. It contains two fragments of that gene plus a short fragment that has so far not been characterized. Scientists think the uncharacterized fragment may be part of the original plant's DNA that got scrambled in the transformation process. It is not clear what this means in terms of safety. But people and animals have eaten Roundup Ready soybeans for five years with no reported ill effects. This finding does indicate that the transformation is not always "clean and precise."

WHAT ARE MARKER GENES?

Scientists need a way to tell if the desired gene has been transferred into the host plant's DNA and if it works. The actual transformation or incorporation of the new gene into the plant's DNA does not happen very often. Transformation is not as efficient as might be desired. Scientists need a way to identify the few plants that actually were transformed from the far larger number that were not. So scientists place a "marker" gene in front of the actual gene to be transferred. Think of the marker and the gene of interest as the head and the tail of a worm. If the head can be detected in the transformed plant, it is more likely the tail was also transferred.

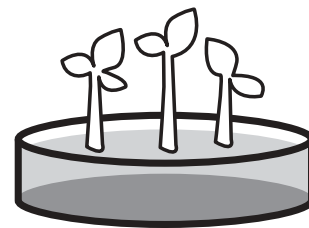
Marker genes are more useful to help the scientist "select" for successful transformation. Many times the successful transformation does not provide plant cells with easily identifiable characteristics at an early stage of growth. So the marker provides a characteristic that can identify those plant cells that were transformed at an early stage, without having to grow the plant. Antibiotic-resistant genes have been used successfully as marker genes. These genes allow plant cells to produce proteins that protect it from the effects of a specific antibiotic. Usually antibiotics inhibit the growth of normal plant cells that are not antibiotic resistant. If an antibiotic marker is used in a plant transformation, the scientist takes the cells exposed to the transformation and grows them in the presence of the antibiotic. Only those cells that received the antibiotic-resistant gene will grow into plants. These plants are more likely to be transformed and can then be tested to see if they received the gene linked to the marker gene. This greatly reduces the amount of testing necessary to identify transformed cells.

WHAT PROBLEMS MIGHT ARISE FROM THE USE OF ANTIBIOTIC MARKER GENES?

Two possible safety issues have been raised with the presence of antibiotic-resistant genes in food crops. One is whether the protein produced by the gene would remain in the food where it could interfere with the effectiveness of orally administered antibiotics. Another is whether the gene could be transferred to bacteria in the intestine and contribute to the growth of antibiotic-resistant strains of bacteria. Tests conducted for FDA approval indicate this protein, as well as its DNA, is rapidly broken down in digestive juices and is not likely to interfere with oral antibiotic use or contribute to antibiotic resistance. Microbiologists in England have demonstrated that it is unlikely that antibiotic-resistant bacteria will arise in the saliva or intestinal tract of animals fed genetically engineered crops. They are also currently investigating whether such resistant strains could form during silage production. However, biotechnology companies are developing other marker genes to phase out the use of antibiotic marker genes.

WHAT IS TISSUE CULTURE?

This is a process in which plant cells or small pieces of plants can be grown in flasks or petri dishes in the laboratory. The dish contains material called media that supplies the nutrients needed by the plant tissue to grow. This technique allows scientists to perform tests on plants more quickly than if working with the entire plant in a greenhouse. It also permits entire plants to be generated from cells or tissue.



DEFINITIONS

- **DNA or deoxyribonucleic acid**—the material in organisms that carries the recipe for making an identical organism. DNA is composed of chemical bases or sub-units strung together to make a double helix.
- **Gene**—a specific set of the bases or sub-units of DNA that contain the information to make one protein.
- **Protein**—a compound that provides structure, physical characteristics or regulates reactions in an organism.
- **Chromosome**—a large sequence of DNA in a structure designed to insure the passage of the organism's recipe to the next generation.

WHERE CAN I LEARN MORE ABOUT GENETIC ENGINEERING?

This publication provides a simplified explanation of genetic engineering. The following Web sites provide more information:

<http://biotech.cas.psu.edu> – this site provides some articles plus a variety of links to other organizations.

<http://www.colostate.edu/programs/lifesciences/TransgenicCrops> – this site explains in more detail (with pictures) how GE plants are created.

<http://www.comm.cornell.edu/gmo> – an excellent site to help you think about genetic engineering applications and their implications.

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Visit Penn State's College of Agricultural Sciences on the Web: <http://www.cas.psu.edu>

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