

## Biotechnology and Genetically Modified Foods

**Introduction:** In this workshop you will create a transgenic plant using the concepts, not the actual technology, of DNA recombination. First, you will evaluate the use of transgenic crops from the perspectives of the consumer and the producer, then you will insert a gene into a bacterial plasmid, to make a new transgenic plant.

### **Key concepts:**

- The traits expressed in an organism can be altered using genes from other organisms. This can be accomplished by DNA recombination.
- Transgenic crops usually have quantifiable economic advantages, but unquantifiable environmental disadvantages.

### **What's due**

1. D2L Workshop 4 "quiz" over terms and concepts p.1-4 - 5 points
2. Chocolate-flavored cherry plasmid DNA p.4-7 - 5 points

This lab has been adapted from 'Dining on DNA'.

See also: <http://www.who.int/foodsafety/publications/biotech/20questions/en/>  
<http://www.newscientist.com/channel/opinion/gm-food/>

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### Introduction

Genetic modification (**GM**) is a relatively new and controversial technique that improves the traits of organisms used for human usage. Originally identified as a genetic process by which genes for antibiotic-resistance were passed from one bacterium to another, the process was soon adopted to artificially produce crops with desirable traits such as pest-resistance. Although the process of **gene-selection** has been used by humans for 10,000 years for crop and animal domestication, and increased **mutation rates** have been used for nearly a century, the GM technique produces **horizontal gene transfer** between unrelated organisms. Potential benefits include unique qualities of organisms and reduced environmental impact from pesticide use. Potential hazards include the potential transfer of genes to unintended organisms, and unforeseen results of this process.

By 2006, 252 million acres of GM crops were being grown in 22 countries; the United States being the global leader, with over half of the global cultivation. Some countries (UK) do not currently permit cultivation of GM crops, but the cultivation of GM crops in developing countries is expected to undergo exponential increase. Over 100 different varieties of 50 different crops are available for cultivation, but some GM crops are no longer cultivated, such as the first GM food, "FlavrSavr tomatoes (DSCS CSU, 2004)"

### Reference Cited

Department of Soil and Crop Sciences Colorado State University. Transgenic Crops Currently on the Market. <http://cls.casa.colostate.edu/TransgenicCrops/current.html>. Last updated March 8, 2004; date viewed February 3, 2008.

Spring 09

## Flavr Savr™ Tomato

In the United States, tomato-lovers spend \$4 billion on tomatoes each year (including salads, pastas, sauces, ketchups, and soups). American consumers expect to be able to purchase fresh tomatoes all year long, so during cold months tomato growers have a hard time keeping up with the demand.

Over the winter, tomatoes grown in southern states are picked while green and shipped to northern states. The tomatoes are then reddened and ripened in containers filled with ethylene gas. Northern consumers complain that ethylene-ripened tomatoes do not have the “backyard summertime” flavor of those available in grocery stores during warm months. Another problem is that because the tomatoes were picked early, they did not receive enough sun and nutrients from the soil to gain vine-ripened flavor and texture. What’s more, ethylene-ripened tomatoes start rotting within 4 –5 days, so many tomatoes spoil before they can be sold.

Pectin, a naturally occurring fiber substance, is what gives tomatoes their firmness. Tomatoes have a gene that codes for an enzyme known as polyG. PolyG actually chews up the pectin in the tomato and causes it to become softer and mushier. Calgene, Inc. genetically engineered a tomato by turning off the gene that codes for polyG. They did this by introducing an ‘antisense’ version of the polyG gene into the tomato. When the antisense gene is introduced, it attaches to the polyG gene, which can then no longer code for the polyG enzyme. The new tomato does not soften as quickly and can stay on the vine longer to gain nutrients and more flavor.

Things to think about:

- Are consumers going to pay more for this tomato?
- Is this a worthwhile use of technology?
- Could this gene transfer affect other traits of the tomato?

## “Golden” Rice

A majority of the world’s nutrient deficiencies concern iron and vitamin-A. Iron deficiency affects approximately 3.7 billion people, most of which are women. Anemia, an illness related to iron deficiency, impairs immunity and reduces physical and mental capacities. Even mild anemia in children can impair intellectual development. Vitamin-A deficiency affects about 7% of the population, mostly children (up to 400 million children). Vitamin-A deficiency makes children vulnerable into infection and worsens the course of many infections. It is also the main cause of blindness among children in developing countries. Each year there are more than one million deaths associated with vitamin-A deficiency.

The important compound in producing vitamin-A is beta-carotene, which is what makes carrots look orange. Rice plants produce beta-carotene, but only in the leafy parts of the plant and not in the rice grain that is consumed by humans. Rice is also high in phytic acid, which is an inhibitor of iron absorption in the human body.

Scientists added three genes to a type of rice to make it produce beta-carotene within the rice grain. Two of these were from the daffodil and one was from a bacterium. The rice grains now contain enough beta-carotene to meet the total vitamin-A requirement of a human diet and, perhaps not surprisingly, they are now orange-colored! To double the iron content in rice, the research team added a ferritin gene from a bean. Ferritin is an iron storage protein found in many animals, plants, and bacteria. They decreased the amount of phytic acid in the rice by introducing a phytase gene that degrades the phytic acid, thus increasing iron absorption during digestion.

The scientists' goal is to distribute the rice free of charge. Local rice breeders can then transfer these new characters of the rice into local varieties using traditional breeding methods.

Things to think about:

- Will people eat orange rice?
- If there is a lot of cross-breeding with local varieties, will we cause some local varieties of rice to go extinct?
- Should we care about these extinctions?

### Roundup Ready™ Soybeans

The most common way farmers control weed problems is to use chemical herbicides, which destroy plants or limit their growth. However, herbicides impact the growth of *all* plants, including the crop plants, and an untimely application of herbicide will kill the crop. Monsanto developed a herbicide called Roundup<sup>R</sup>, which has glyphosate as its active ingredient. Glyphosate is called a 'broad spectrum herbicide' because it negatively impacts many different types of plants; but it is also a relatively benign herbicide because it degrades quickly.

Monsanto then identified a gene that enables plants to tolerate Roundup<sup>R</sup> herbicide. They transferred this gene into soybean plants, and market these as Roundup Ready™ Soybeans. These soybeans are resistant to applications of the Roundup<sup>R</sup> herbicide, which allows farmers to freely apply Roundup<sup>R</sup> without harming the soybean crop. Monsanto charges a 'technology fee' when farmers buy the Roundup Ready™ seeds, and the farmers are obligated to use Monsanto's Roundup<sup>R</sup> herbicide. Traditionally, farmers have kept some of their crop and used this seed to plant the following year; Monsanto does not allow farmers to do this with seeds produced by Roundup Ready™ soybeans.

Things to think about:

- What happens if the Roundup Ready™ gene gets into another plant, like some sort of weed that grows near the field? There are many plants that are wild relatives of soybeans.
- Will farmers increase the use of Roundup<sup>R</sup> herbicide because they know that it won't hurt their crop?
- Are Monsanto's policies fair to the farmers?
- If you eat any products with soya lecithin as an ingredient or that have been fried in soy oil, you've eaten genetically altered soybeans!

### Bt cotton

One of the greatest problems that many farmers face is the loss of crops due to insect damage. Insects may attack different parts of the plant (leaves vs. stems vs. fruits) and at different times during plant growth. Chemical pesticides need to be applied throughout the growing season to reduce these losses.

*Bacillus thuringiensis*, Bt, is a bacterium that occurs naturally in the soil. When some insects ingest a protein produced by Bt, the function of their digestive systems is disrupted, producing slow growth and ultimately, death. Bt is only effective against certain insects, including European corn borers and cotton, Colorado potato beetles, and certain flies and mosquitoes.

Scientists isolated the gene in Bt that produces the protein that kills these insects. The gene has been transferred – as a plasmid – to watercress, corn, cotton, and other plants. Transgenic plants containing this gene express it in ways that are very different than the bacteria would. Therefore, synthetic genes have been created that will “switch on” Bt production in fruit, the green tissue, or other parts of the plant.

Studies have shown that farmers using Bt cotton have significantly higher crop yields and use fewer insecticides. And, crops in nearby fields benefit from growing Bt cotton (Stone, 2008). However, Bt cotton is more susceptible to drought conditions, and yields during dry periods lower than those of non-Bt cotton. Moreover, the moths that feed on Bt-watercress have become resistant to the Bt protein.

#### Reference Cited

Stone, R., 2008. GM crops make good neighbors. *Science News* Sept. 18. Available from <http://sciencenow.sciencemag.org/cgi/content/full/2008/918/3?etoc> Viewed Sept. 20, 2008.

Things to think about:

- Are there insects besides plant pests that might be affected by Bt-cotton? How might they be exposed to the Bt in the transgenic plants?
- In warm climates, insects may go through several generations during a single growing season, increasing the likelihood that they will develop resistance. What might happen if the bollworm develops resistance to Bt cotton?

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## Creating a transgenic organism (Plasmid exercise)

### Horizontal Gene Transfer

Ordinarily, genes are passed from one generation to the next, but single-celled organisms can transfer genes from one organism to another. This is one reason antibiotic resistance spreads so rapidly among bacteria. DNA can be moved from one bacterium to another by viruses in a process known as “**transduction**,” or through the process of “**transformation**,” in which DNA is passed from one bacterium to another. For example, in 1973, it was discovered that the bacterium *Escherichia coli* had acquired a gene for antibiotic resistance from the bacterium *Salmonella typhimurium*. Geneticists then developed techniques to use this property and cause bacteria (called **vectors**) to pass desired genes from one organism to another.

There are now several techniques used to accomplish horizontal gene transfer including chemical and electrical techniques in addition to bacterial and viral **vectors**. But, the earliest was “**plasmid transformation**” in which a desirable gene was isolated from a host, inserted into the circular DNA (the **plasmid**) of certain bacteria, which then was transfer to the target organism. In order to produce a genetically-modified organism, the gene is transferred to an embryo, which then develops into a seed-producing adult with the desired genetic traits.

Firm tomatoes, nutritious rice, herbicide-resistant soybeans and pest-resistant cotton are real examples, described above. In many cases, more than one gene controls a desired trait, and discovering gene sequences can be extremely difficult.

Situation (completely imaginary):

A large candy company has hired your laboratory to conduct a very important project. The company is attempting to develop a new product, chocolate flavored cherries. Consumer surveys indicate that people love the combination of chocolate and cherries and the ACME Candy Company wants to be the first to put these delicious morsels on the market. You are the laboratory technician given the task of altering the DNA of a cherry tree so that it bears a fruit that has a chocolate flavor to it. The scientist in your laboratory has isolated a gene in the cacao bean that codes for the delicious chocolate flavor. It is your job to remove this gene from the cacao bean and insert it into the cherry seedling so that you end up with the new chocolate flavored cherry.

You will need to know the following terms:

**ligase** – an enzyme which binds the loose ends of genetic strands together.

**plasmid** – a circular ring of DNA which is found in some bacterium.

**restriction enzyme** – a specific molecules that cuts the DNA in specific places.

**transgenic** – refers to moving genes from one species to another

**vector** – an agent used to transfer genetic information from one organism to another.

Examples include viruses and bacteria (with plasmids).

<p><b>Materials:</b> printout of p. 7, scissors, tape.</p>
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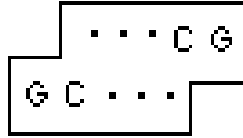
**The following instructions refer to the diagrams on page 7**

Methods:

A. Removing the desired gene from the linear cacao DNA

Pick up your restriction enzyme.

1. Beginning on the top of your cacao DNA ladder at the end that indicates 'start' read the bases of the strand until you have read an **AGCT** sequence all in a row in that order.
2. Use your restriction enzyme to make a cut between the A and the G in the four base sequence. (only cut half-way through the DNA strand)
3. Continue to make cuts after the A in every four-base AGCT sequence.
4. Now begin reading the DNA on the bottom strand of your cacao DNA ladder. Start reading from the end that indicates 'start' and look for an AGCT sequence all in a row in that order.
5. As before, use the restriction enzyme to make a cut after the A (half-way through between the A and the G) in every four base AGCT sequence.
6. One cut on the top cacao DNA strand should be two bases away from one cut on the bottom cacao DNA strand. Cut through the hydrogen bonds connecting the bases of the DNA strand to connect the two closest cuts.
7. Repeat this step on the opposite end of the DNA ladder. You should make a total of two cuts down the middle of the ladder, right through the hydrogen bonds.
8. Remove the strip of DNA that comes out of the DNA ladder. This piece of DNA should have two exposed rungs and a central portion of the ladder intact. It contains the chocolate-flavor gene and should be shaped like this:



- B. Getting the plasmid ready for insertion of the gene
1. Cut your circular plasmid out so that it looks like a large doughnut ring.
  2. Beginning on the outside at the arrow, start reading along the plasmid in the direction of the arrow until you come across an AGCT sequence all in a row.
  3. With your restriction enzyme, make a shallow cut after the A in every AGCT sequence.
  4. Now going in the opposite direction read along the inside loop of the plasmid, reading until you come across the AGCT sequence on the inside DNA strand.
  5. With your restriction enzyme, make a shallow cut after the A in every AGCT sequence
  6. Once again, each cut on the inside loop should be two bases away from a cut on the outside loop.
  7. Cut through the hydrogen bonds right down the middle of the plasmid loop in order to connect each of the two closest cuts.
  8. With the final cut, open the loop and look closely at the two exposed bases.
- C. Insertion of the new gene into the plasmid (Recombination)
1. Compare the strip of DNA that you removed from the cacao DNA with the cut-open plasmid DNA. Can you see how they match together? The two pieces of DNA fit together like a puzzle.
  2. Match the shapes as well as the bases (A with T, C with G across the rows of the plasmid DNA and the cacao DNA)
  3. Use your ligase to insert the cacao DNA into the plasmid loop.
  4. Tape the plasmid carefully onto a piece of paper.

WRITE YOUR NAME ON THE BACK OF THE PLASMID and hand it in, in class on the due date.

