

Transformation

Transgenic plants (plants that have had genes from another type of organism inserted into them) are commonplace in agriculture today. More than 75 percent of the processed food in the United States is derived from genetically engineered organisms.¹ Pest resistance is one of the most common traits introduced into plants through genetic engineering. Other examples of transgenes include genes for increased growth, longer shelf life, and increased flavor.

Although there are benefits to transgenic plants such as increased crop yield or increased pest resistance, which were just mentioned, there are also concerns about the safety of those plants. One concern is the possibility that genetically engineered plants may breed with their traditional counterparts. Although it is possible to genetically test individual plants to determine if they have been genetically engineered, it is not economically feasible to test every plant from an entire field of crops.

How would farmers know for sure that the plants they grow each year were, or were not, genetically engineered? Scientists have come up with a possible solution to this problem. Insertion of a **reporter gene** along with the gene that contains the desired transgenic trait (such as the ability to resist a certain pest species) would ensure that all plants that were genetically engineered would also produce a discernable phenotypic trait.² A reporter gene is a gene that produces some sort of phenotypic signal.

Scenario: Scientists are inserting a gene for pest resistance (**Bt gene**) into soybeans. The Bt gene naturally occurs in a species of bacteria. It codes for a protein that is toxic to certain insects. By inserting this gene into a crop, a farmer can make that crop resistant to insects.

This transgenic plant containing the Bt gene will be sold to a group of farmers whose fields are next door to a farmer who grows only organic crops. The organic farmer has expressed concern that some of the transgenic soybeans from his neighbor will accidentally be sown in his organic fields. To mitigate this problem, the farmer planting the transgenic crop has agreed to use a species that contains a reporter gene in addition to the Bt pest resistance gene.

In this activity, you will help the scientists design a **plasmid** that will contain the gene for pest resistance (Bt gene), as well as a reporter gene (green fluorescent protein gene). Once you have inserted the genes of interest in your plasmid, your research and development team will take over and continue the transformation process that will insert the plasmids into bacteria known as **Agrobacterium tumefaciens**. Plants will then be infected with the transformed bacteria, thus delivering the genes for pest resistance and green fluorescent protein into the soybeans.

Transformation (continued)

Often, reporter genes involve a trait such as antibiotic resistance that allows scientists in the lab to select successful transformants by growing them in the presence of antibiotics that will kill any plants that did not successfully incorporate the desired trait. But another type of reporter gene can cause an organism to glow in the presence of ultraviolet light. This type of trait is visible to the naked eye and would allow farmers to know, simply by looking at a plant at night using an ultraviolet light, whether or not it has been genetically engineered.

The type of gene that researchers use for this detection is one that codes for (that is, has a DNA sequence that directs the production of a specific protein) a green fluorescent protein and is referred to as a **GFP gene**. The most common source of this gene is marine organisms such as jellyfish that produce the protein naturally.²

Endnotes:

1. "Exploring the Moral and Ethical Aspects of Genetically Engineered and Cloned Animals," Pew Initiative on Food and Biotechnology, January 2005, http://pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Summaries_-_reports_and_pubs/PIFB_Moral_Ethical_Aspects_GE_and_Cloned_Animals.pdf.
2. C. N. Stewart, "Monitoring the Presence and Expression of Transgenes in Living Plants," *Trends in Plant Science* 10, no. 8 (2003): 390-96.

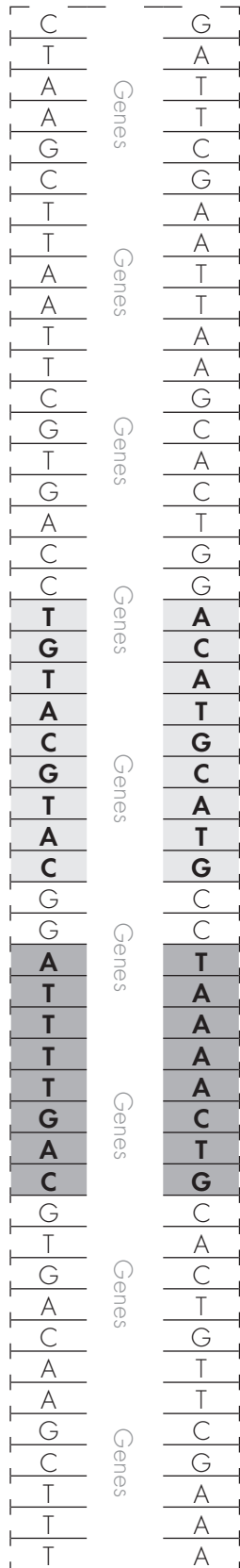
STUDENT PAGE

Paper Plasmid Construction

1. Cut out each strip of DNA on the dotted lines.
2. Tape the strips together in any order (but make sure the letters are facing the same way).
3. This is your paper representation of a plasmid. A plasmid is a circular piece of DNA that is extrachromosomal (that is, is not part of the DNA found in an organism's nuclear DNA).

A	T	C	G	C	G	T	A
G	C	G	C	T	A	T	A
T	A	A	T	G	C	C	G
C	G	T	A	C	G	G	C
A	T	G	C	A	T	C	G
G	C	G	C	G	C	A	T
A	T	A	T	G	C	G	C
G	C	T	A	C	G	T	A
G	C	G	C	A	T	T	A
T	A	A	T	G	C	T	A
T	A	A	T	A	A	G	C
C	G	A	T	C	G	G	C
C	G	T	A	C	G	A	T
A	T	T	A	C	G	A	T
G	C	C	G	G	C	A	T
G	C	A	T	T	A	A	T
T	A	T	A	A	T	G	C
A	T	T	A	A	T	C	A
G	C	T	A	T	A	T	A
G	C	T	A	G	C	T	A
C	G	T	A	T	A	A	T
A	T	A	T	G	C	T	A
T	A	G	C	A	T	G	C
G	C	C	G	G	C	T	A
T	A	C	G	T	A	C	G

Genes for Insertion into Plasmid



This strip of DNA contains copies of both the GFP gene (bold and on dark gray) and the Bt resistance gene (bold and in light gray). The two genes were generated using polymerase chain reaction and pieced together using an enzyme known as ligase. Cut out the strip of DNA along the dotted line. This fragment of DNA will be the one you will insert into the plasmid.

Note that the sequences have been greatly reduced in length for this simulation. The true gene sequences would be hundreds of base pairs in length.

Restriction Enzymes

The following diagram (Figure 1) illustrates the specific sequences recognized by three different **restriction enzymes**: **EcoRI** (pronounced "eco ar one"), **HindIII** (pronounced "hin dee three"), and **PstI** (pronounced "pee ess tee one"). The cutting site is indicated with scissors. Each of these sequences is a **palindrome**. (Hint: The words "civic," "level," and "radar" and the phrase "a man, a plan, a canal: Panama" are palindromes.) Why do you think this sequence occurs?

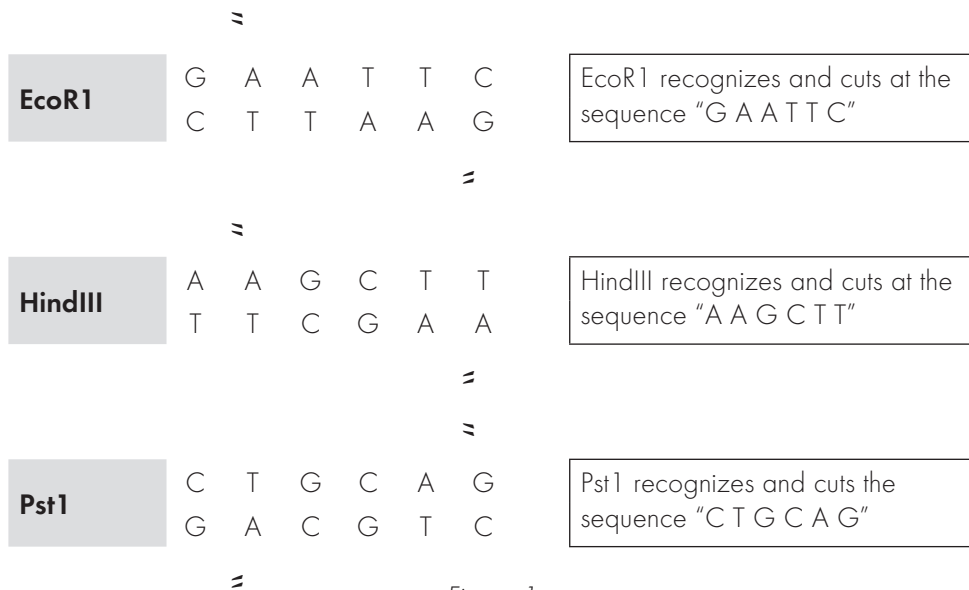


Figure 1

Because each of the three restriction enzymes cuts within the recognition sequence, "**sticky ends**" are produced. A sticky end is a single strand of overhanging base pairs that allow two sequences to be aligned and joined by the enzyme ligase (see Figure 2).

Your job is to decide which restriction enzyme you would use to cut out the genes that will be inserted into your plasmid and to cut your plasmid to allow the insertion. You will use the same restriction enzyme for both. Keep the following in mind when choosing your restriction enzyme:

- You want to cut the plasmid in only ONE location.
- You will need the restriction enzyme to cut both ends of the genes you will insert. This change will create sticky ends that will allow the insert to be ligated into the plasmid.

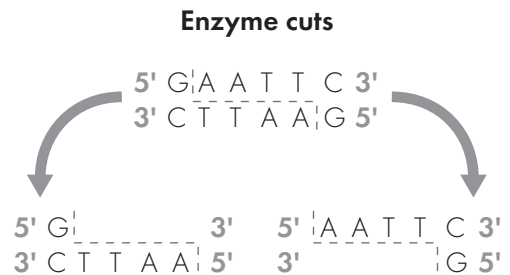
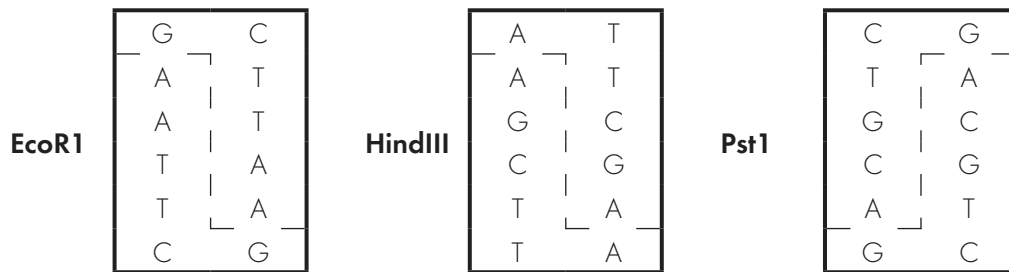
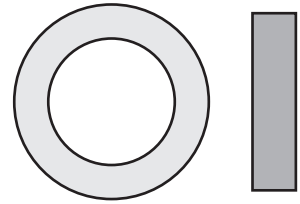


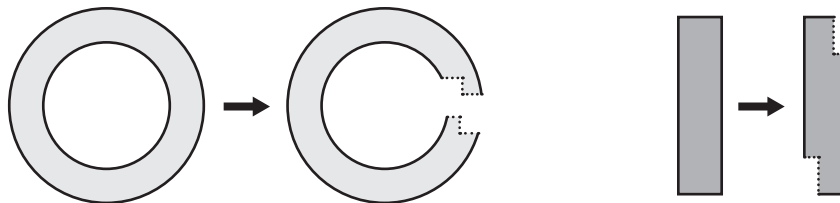
Figure 2. Example of an EcoRI-Cutting Site that Produces "Sticky Ends"

Instructions

1. Cut out the strips of DNA from the page labeled "Paper Plasmid Construction." Tape them together (in any order, as long as the letters face the same way) to form a circle. This circle represents your plasmid DNA.
2. Now, cut out the piece of paper that represents the genes you are going to insert from the page labeled "Genes for Insertion into Plasmid." This paper represents the two genes that you are going to insert into your plasmid.
3. Next, you need to identify which restriction enzyme to use that will produce the correct cutting pattern. You want the restriction enzyme to cut the **plasmid** only once, but it needs to cut the piece of DNA that contains the **genes** you want to insert twice. Below, circle the restriction enzyme that is the best choice, and circle the cutting site on both the plasmid and gene. Use Student Page: Restriction Enzymes to learn more about how restriction enzymes work.



4. Once you have identified the appropriate restriction enzyme, use your scissors to cut both your plasmid and your gene at the restriction enzyme cutting sites (follow the dotted lines on the restriction enzyme you circled above). Your results should look like the pictures below.



5. Once you have made the appropriate cuts with scissors, you are ready to insert the genes into the plasmid. Tape the genes into the plasmid, making sure to match up the corresponding base pairs (remember, A pairs with T, G pairs with C). Your final product should be a complete plasmid with two novel genes inserted.

