

## Terminator Technology

Having invested considerable resources into the production of genetically engineered plants it is often beneficial to prevent the formation of viable seeds that contain the genetically engineered genes. This can be illustrated by examining the production of soybean seeds. For example, the company Monsanto has developed glyphosate resistant soybean plants/seeds. Glyphosate is an herbicide that kills plants by inhibiting the plant version of Enol Pyruvate Synthase (EPS), which is needed for the production of some of the aromatic amino acids. The bacterial version of EPS is not inhibited by glyphosate (or certainly not as much as the plant version). Monsanto has made transgenic soybean plants that contain the bacterial version of Enol Pyruvate Synthase (EPS). They were made available in 1996 and within less than 10 years over 85% of all soybean fields in the US were planted with glyphosate resistant seeds. A farmer buys the seeds, plants the seed and then at harvest time they harvest the bean kernels/seeds. These seeds are glyphosate resistant, and rather than buy new seeds from Monsanto next season, the farmer could simply plant some of this year's crop next season, unless some special modification was taken to prevent those seeds from being able to germinate. Remember, Monsanto is in the business of selling seeds.

A farmer buys seeds and plants the crop. Consider those plants to be the F1 generation. These plants produce seeds that are the F2 generation. If the F2 seeds were viable then the farmer could plant some of their harvest and they would have glyphosate resistant plants. You don't want to affect seed production in the parental plants since it is the soybean seed that is harvested and sold by the farmer. You need to find a way to allow seed production so the farmer can sell their harvest, yet prevent seed germination if the farmer were to plant their harvested seeds. Terminator technology is the general term used to describe this approach. As illustrated in lecture it can be complicated. Monsanto must control the technology to prevent seed germination by the farmer. It would be relatively easy to kill developing plants, but Monsanto must also be able to grow their own plants to produce the seeds they sell, then make sure the farmer cannot do the same. Thus they need to design a system that automatically prevents germination in the farmer's harvested seed, yet allows Monsanto to produce seeds to sell. The general approach is diagrammed below.

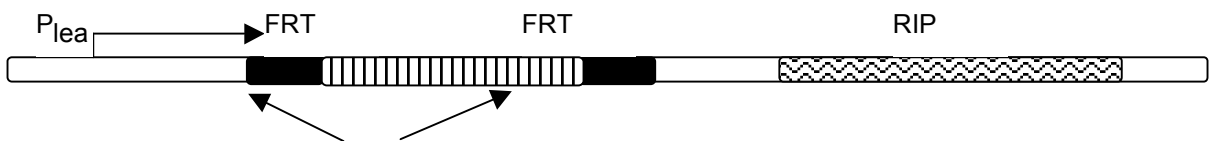
There will be three components to the system. The first component that will be discussed is the mechanism that prevents seed germination (i.e. termination). The second component that will be discussed is the system that controls when termination occurs. The final component is a system that allows Monsanto to control the timing of termination so they can generate seeds that they can sell.

Refer to the following diagram.



$P_{lea}$  stands for a promoter that is activated late in embryonic development. Don't forget that when the seed develops there is an embryo inside it. FRT represents a specific DNA sequence that is recognized by a specific DNA recombinase (this enzyme will be discussed later). RIP is a gene that encodes a Ribosome Inhibiting Protein. If RIP is expressed within a cell, the cell dies, and hence in this case the developing embryo dies. However,  $P_{lea}$  must be adjacent to the RIP gene for RIP to be expressed. (This was not made clear in lecture.) Without recombination, RIP is not expressed due to the large intervening sequence between the FRT's.

If a specific recombinase that recognizes FRT is present, then there is INTRAMOLECULAR recombination. This recombination removes the large intervening sequence between the FRT's. Notice that the recombination is NOT occurring during meiosis, but occurs during the mitotic cell cycle. This is illustrated below in two illustrations. The arrows indicate the position of recombination before recombination. Note the placement of the promoter after recombination.



**Before recombination.**



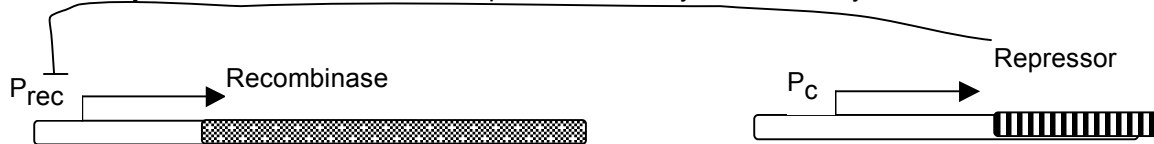
**After recombination.**

Note RIP is now under the control of  $P_{Iea}$ . Since the promoter is specific for late embryonic development the seed develops fine but the embryo does not. Thus you don't affect seed development much, except for the absence of a viable embryo. Also note that this change is permanent: the piece of DNA containing the intervening sequence is lost during cell division (it lacks an origin of replication and centromere), and it is impossible for it to re-insert into the chromosome.

#### Controlling the activity of recombinase

There is a second genetic locus that encodes a recombinase that is under the control of a specific promoter ( $P_{rec}$ ). When the promoter is active, the recombinase gene is transcribed, the mRNA is translated and the protein product, recombinase, can function by catalyzing intramolecular recombination at FRT sites. There are several possible approaches for controlling the promoter. If the promoter is constitutive then recombinase is constitutively produced. Monsanto, however, needs to inactivate the recombinase when they are producing seed so that the seeds they produce have viable embryos. Monsanto could apply a chemical that binds to the promoter and blocks transcription of recombinase. However, this would require that they apply this chemical at every generation; if they forgot to do so, they would never be able to recover the line again—it would be dead.

The approach they used was to introduce an additional gene that produces a repressor that binds at the  $P_{rec}$ . The repressor is under the control of constitutive promoter ( $P_C$ ). Therefore, the repressor protein is made constitutively, no recombinase is made, and no recombination occurs and seeds are produced with viable embryos. This allows Monsanto to produce as many seeds as they want to continue their line.



When Monsanto is ready to sell seeds, they need to activate the recombinase by treating their harvested seeds with a chemical that turns on the recombinase in the seed being sold to the farmer. The chemical blocks the repressor from binding to the  $P_{rec}$ . Thus when those seeds grow into plants they contain cells that contain DNA which has no intervening sequence between  $P_{Iea}$  and RIP (due to recombination). The plants grow fine and make fairly normal developed seeds but the seeds produced by that plant do not have viable embryos. Why? Because only the  $P_{Iea}$  has the RIP gene, and that promoter is active only in embryonic cells and only during late embryonic development. Hence, the seeds develop fairly normal but lack viable embryos. The farmer can sell their crop but the seeds are not viable.

To summarize, because the  $P_{Iea}$  promoter driving the RIP gene is active late in seed development, but before embryo development is complete, Monsanto can treat fully developed seeds with the chemical to allow the recombination event to occur, but RIP will NOT be expressed in those seeds because the time for  $P_{Iea}$  expression is over for that generation. When those seeds are planted, they grow into healthy adult plants which contain cells that have undergone intramolecular recombination. When it comes time for the plants to produce seed the RIP gene is expressed. Seeds develop fine except for the absence of a viable embryo. Because the recombination is permanent, there is nothing the farmer can do to make the seeds viable.

On a side note I imagine there is currently research being conducted to generate glyphosate resistant cocoa, cannabis, poppy plants, etc. as glyphosate is quite commonly used to eradicate these plants. This research, if being done, is probably not widely advertised.