Crop Biotechnology: Growing and Testing Roundup Ready Soybean Kit

Principal Concept:

This activity introduces the audience to concepts of crop biotechnology and the use of biotechnology to help farmers as it relates to crop yield and weed management. Weed management is an important factor in agricultural production that impacts crop yield or the amount of a crop that is produced. Weeds that are not controlled will reduce the quantity of a crop that a farmer can produce since weeds compete with planted crops for available nutrients and water. Biotechnology is one of many tools helping farmers produce more food, fuel and fiber while reducing their agricultural footprint.

Roundup Ready soybean was developed in 1990 using biotechnology. To produce Roundup Ready soybean, scientists isolated the 5-enolpyruvylshikimate-3-phosphate synthase gene (cp4 epsps) from a naturally occurring microbe (Agrobacterium sp. strain CP4) and through particle gun bombardment plant transformation and inserted the gene into the genome of soybean (Glycine max). The cp4 epsps gene allows soybean to produce the CP4 EPSPS enzyme that confers tolerance to the herbicide glyphosate, commercially known as Roundup. Roundup Ready soybean (event 40-3-2 or MON-04032-6) have been grown on over 1.8 billion acres since it was introduced to farmers in 1996.

Roundup Ready soybean was developed to help farmers manage weeds in their fields. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75%. Nearly all soybean fields receive some type of herbicide treatment; Monsanto scientists developed the Roundup Ready soybean technology as a tool to help farmers control their weeds in soybean fields.

Roundup has been used since the 1970s and has been shown to be safe both for the environment as well as in food and feed crops soybean when sprayed according to label directions. Due to Roundup Ready soybean’s tolerance to glyphosate (via the CP4 EPSPS enzyme), Roundup can be directly applied to soybean fields.

This biotech kit contains Roundup Ready and conventional soybean seed as well as background information on soybean and how Roundup Ready soybean was invented.

Students can perform several activities:

1. Growing Roundup Ready and conventional (i.e. non-genetically modified) soybeans

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2. Comparing of growth patterns between Roundup Ready and conventional soybeans
3. Demonstrating of Roundup Ready weed control technology
4. Detecting the Roundup Ready protein by Quickstrip Test (lateral flow immunoassay) and DNA detection Roundup Ready insert by polymerase chain reaction (PCR).

**Grade Levels:**
This activity is appropriate for grades 6-12.

**Performance Standards:**
This project meets the following Next Generation Science Standards:

MS-PS1-2: Analyze and interpret data on the properties of substances before and after the substances interact to determine if a chemical reaction has occurred.

PS1.B: Substances react chemically in characteristic ways. In a chemical process, the atoms that make up the original substances are regrouped into different molecules, and these new substances have different properties from those of the reactants.

MS-LS1-5: Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.

LS1:B: Genetic factors as well as local conditions affect the growth of the adult plant.

MS-LS4-5: Gather and synthesize information about the technologies that have changed the way humans influence the inheritance of desired traits of organisms.

LS4:B: In artificial selection, humans have the capacity to influence certain characteristics of organisms by selective breeding. One can choose desired parental traits determined by genes, which are then passed on to offspring.

HS-LS2-7: Design, evaluate and refine a solution for reducing the impacts of human activities on the environment and biodiversity.

LS4.D: Biodiversity and Humans

Biodiversity is increased by the formation of new species (speciation) and decreased by the loss of species (extinction). *(Secondary to HS-LS2-7)*

Humans depend on the living world for the resources and other benefits provided by biodiversity. But human activity is also having adverse impacts on biodiversity through overpopulation, overexploitation, habitat destruction, pollution, introduction of invasive species, and climate change. Thus sustaining biodiversity so that ecosystem functioning and productivity are maintained is essential to supporting and enhancing life on Earth. Sustaining biodiversity also aids humanity by preserving landscapes of recreational or
inspirational value. (secondary to HS-LS2-7) (Note: This Disciplinary Core Idea is also addressed by HS-LS4-6.)

ETS1.B: Developing Possible Solutions

When evaluating solutions it is important to take into account a range of constraints including cost, safety, reliability and aesthetics and to consider social, cultural and environmental impacts. (secondary to HS-LS2-7)

ETS1.A: Defining and Delimiting Engineering Problems

Criteria and constraints also include satisfying any requirements set by society, such as taking issues of risk mitigation into account, and they should be quantified to the extent possible and stated in such a way that one can tell if a given design meets them. (HS-ETS1-1)

Humanity faces major global challenges today, such as the need for supplies of clean water and food or for energy sources that minimize pollution, which can be addressed through engineering. These global challenges also may have manifestations in local communities. (HS-ETS1-1)
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Glycine max (Soybean) and Development of Roundup Ready Soybean

**Roundup Ready Soybean**

Roundup Ready soybean was developed by the Monsanto Company in 1990 using biotechnology. To produce Roundup Ready soybean, scientists isolated the 5-enolpyruvylshikimate-3-phosphate synthase gene (*cp4 epsps*) from a naturally occurring microbe (Agrobacterium sp. strain CP4).

The *epsps* gene encodes the EPSPS protein, which is an enzyme that catalyzes the production of aromatic amino acids in plants. Those are essential for plant growth. The enzymatic activity of EPSPS in plants (e.g. weeds) can be inhibited by spraying a glyphosate-based herbicide (such as the commonly used herbicide Roundup). If EPSPS is inhibited, the plants stop producing these essential amino acids and eventually die. Using recombinant DNA techniques and, particle gun plant transformation, Monsanto scientists stably inserted the *cp4 epsps* gene into the genome of soybean (*Glycine max*). The *cp4 epsps* gene allows soybean to produce the CP4 EPSPS enzyme, which is a version of the EPSPS enzyme that is tolerant to glyphosate. The presence of CP4 EPSPS confers glyphosate-tolerance to the soybean plants.

Monsanto sought relevant regulatory approvals for Roundup Ready soybeans and launched the product in 1996. The ability for farmers to use glyphosate over the top of their soybean crop was revolutionary. It significantly simplified their weed control practices and production system. Roundup Ready soybeans (event 40-3-2) have been grown on over 1.8 billion acres since it was introduced to farmers in 1996.

**Why Develop Roundup Ready Soybean?**

When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75%. Nearly all soybean fields receive some type of herbicide treatment. Monsanto scientists developed Roundup Ready soybean as a tool that farmers can use to control their weeds in soybean fields.

Roundup has been used since the 1970’s and has been shown to be environmentally safe and safe to use on food and feed crops like soybean when sprayed according to label directions. Roundup is a very effective herbicide and can be applied directly to Roundup Ready soybean because the soybean is tolerant to Roundup herbicide due to the production of the CP4 EPSPS enzyme. Using glyphosate on growing soybean crops without impacting the soybeans brought many benefits to growers. It also allowed the use of reduced tillage agricultural production systems that, in turn, conserve the valuable, fertile top soil on agricultural fields.

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1Roundup Ready soybean were one of the first genetically modified crops produced and [not sure what trying to say here] utilized particle gun transformation. Most of the crops now on the market were produced through Agrobacterium based plant transformation.
Soybean History and Uses
In early recorded history, Chinese farmers used soybeans for the preparation of various types of soy foods, including soymilk, tofu, soy sauce, and soy paste, and had started to consume soybean sprouts by themselves (Liu, 2004). Domestication of soybean is thought to have taken place in China during the Shang dynasty (approximately 1500 to 1027 B.C.) or earlier (Hymowitz, 1970).

From the first century A.D. to approximately the 15th and 16th centuries, soybean was introduced into several countries, with locally adapted soybean varieties, called land races, eventually developing in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal, and Northern India. The movement of soybean throughout this period was due to the establishment of sea and land trade routes, the migration of certain tribes from China, and the rapid acceptance of seeds as a staple food by other cultures (Hymowitz and Newell, 1981; Hymowitz et al., 1990). Starting in the late 16th and throughout the 17th centuries, soybean was used by the Europeans, and in the 17th century, soybean sauce was a common item of trade from the East to the West.

Soybean was introduced into North America in the 18th century. In 1851, the soybean was introduced in Illinois and subsequently throughout the U.S. Corn Belt. In 1853, soybean seed were deposited at the New York State Agricultural Society, the Massachusetts Horticultural Society, and the Commissioner of Patents. The two societies and the Commissioner of Patents sent soybean seed to dozens of growers throughout the U.S. Soybean has been cultivated extensively and improved through breeding following its introduction in the U.S. and subsequently has become a key source of nutrients for food and feed use in the U.S. (Hymowitz and Singh, 1987).

Soybean is now grown as a commercial crop in over 35 countries. In the U.S. soybean was planted on approximately 75 million acres in 2011, producing 3.06 billion bushels2 of soybean with a value of $35.7 billion (ASA, 2012). The major producers of soybeans are the U.S., Brazil, Argentina, China, India, Paraguay, and Canada, which accounted for approximately 95% of the global soybean production in 2011. Approximately 33% of the 2011 world soybean production was produced in the U.S (ASA, 2012). The United States exported 34.7 million metric tons (MMT) of soybeans, which accounted for 37% of the world's soybean trade. Approximately 44 MMT of soybeans were crushed in the U.S. in 2011 and used to supply the feed industry for livestock use or the food industry for edible vegetable oil and soybean protein isolates (ASA, 2012). U.S. stock levels were 7.5 MMT at the end of the year (ASA, 2012).

Taxonomy
Cultivated soybean, Glycine max (L.) Merr., belongs to the family Leguminosae, the subfamily Papilionoideae, the tribe

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2 A bushel is an ancient unit of measure used to standardize trade and measure the productivity of a field. It was formerly used as a measure of volume but is now based on mass or weight. One bushel of soybean weighs 60 lbs.
Phaseoleae, the genus *Glycine* Willd., and the subgenus *Soja* (Moench) F.J. Herm.

**Family:** Leguminosae  
**Subfamily:** Papilionoideae  
**Tribe:** Phaseoleae  
**Genus:** *Glycine*  
**Subgenus:** *Soja* (Moench) F.J. Herm.  
**Species:** max

The genus *Glycine* Willd. is of Asian and Australian origin and is divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* consists of 22 wild perennial species, which are indigenous to Australia, West, Central and South Pacific Islands, China, Russia, Japan, Indonesia, Korea, Papua New Guinea, the Philippines, and Taiwan (Hymowitz, 2004). The subgenus *Soja* includes the cultivated soybean, *G. max* (L.) Merr. and its wild annual relatives from Asia, *G. soja* Sieb. and Zucc.

**Soybean Growth and Development**

Soybean growth stages are designated by two characters: “V” for vegetative, or “R” for reproductive. Some of the vegetative stage designations are VE for emergence, and V1 through Vn for the appearance of the sets of three-part leaves. There are eight reproductive stages: R1 for the appearance of the first flower, R3 for beginning of pod development, R5 for beginning of seed development, and R8 for full maturity (Pedersen, 2008); (Heatherly and Elmore, 2004). Soybeans are typically planted in May to June in the Midwest and are harvested in September to October.

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**Figure 1.** Soybean growth and development stages.  
Source: http://weeds.cropsci.illinois.edu/extension/Other/POCKETcrop.pdf

**Soybean Variety Development**

Crop domestication and improvement through breeding has been largely achieved through selection of genes that regulate the expression of desirable traits, such as those associated with higher yields or disease resistance. Once plants with the desired traits have been selected, a population of those plants with similar characteristics is classified as a variety. Historically breeders have developed desirable varieties by holding onto those desirable plants for further breeding. In recent years, breeders have used the more direct methods of molecular breeding techniques, such as marker assisted breeding, to accelerate the process of identifying breeding lines containing a desired set of positive traits. These techniques rely inspecting the plant’s genetic code for portions associated with desirable traits. Once those portions of code have been identified, molecular breeders can

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3Molecular breeding is the application of molecular biology tools to help plant breeders develop and maintain new plant varieties. The use of these techniques has greatly reduced the time it takes to develop new plant varieties. For additional information see: https://www.isaaa.org/resources/publications/pocketk/19/default.asp.
quickly select the offspring inheriting the genes for further development and testing in the field (Voosen, 2009).

Hundreds of soybean varieties are tested for field performance each year in the U.S. in trials conducted by universities and private companies in all the major soybean growing states. Some of the conclusions that come from variety trials can include maturity group, disease resistance, yield, maturity date, percent lodging (plants fallen over on the ground), height, and herbicide resistance (Tylka et al., 2010). In different parts of the country and/or in other trials, additional characteristics may be identified, such as iron deficiency tolerance or protein or oil content (Pedersen, 2008).

Soybean growth is dependent on day/night length; therefore, different varieties are developed for different latitudes. In the U.S. ten geographically-designated “maturity groups” originally defined by Scott and Aldrich (Scott and Aldrich, 1970) are widely used (Zhang et al., 2007). These maturity groups are mapped as bands extending from north to south, beginning with Group 00 at the far north and ending with Group VIII in the far south (See Figure 2). Groups II, III and IV, which extend from approximately the northern border of Iowa to the southern tip of Illinois, account for approximately 76% (24%, 36%, and 16%, respectively) of the soybean planted in the U.S. Because longer day length delays maturity, a soybean cultivar suited to a southern maturity group would mature too late if planted too far north. Conversely, a northern cultivar would mature too early if planted in the south (Heatherly and Elmore, 2004).

Maturity groups are often designated by Arabic rather than Roman numerals, so the sequence is 00 to 8, and there are subdivisions within the major maturity groups. These are designated by a decimal value. For example, a variety with maturity group designation 2.9 would be at the southern end of Group II.

![Figure 2](http://corn.agronomy.wisc.edu/Crops/Soybean/L001.aspx)

**Figure 2.** Soybean varieties are designated by numbers ranging from 00 to IX (also know as maturity groups). These groups are developed for optimum growth north to south, primarily based on day length. Source: http://corn.agronomy.wisc.edu/Crops/Soybean/L001.aspx

**Uses of Soybean**

Soybean has the remarkable ability to produce more edible protein per acre of land than any other known crop (Liu, 2004). On average, dry soybean contains roughly 40% protein and 20% oil, and it has the highest protein content among cereals and other legume species, and has the second-highest oil content among all food legumes (Figure 3).
Figure 3. Seeds are the reproductive mechanism of plants. They contain a seed coat surrounding an embryo and stored food to help the next generation grow. Soybean seeds contain oils (fats) as well as protein and other compounds stored in their seeds. Source: U.S. Soybean Board [https://www.flickr.com/photos/unitedsoybean/16153987938](https://www.flickr.com/photos/unitedsoybean/16153987938)

Soybean is highly versatile and can be processed into a wide variety of food products including tofu, soybean sauce, soymilk, energy bars, and meat substitutes. A major food use for soybean is purified oil, for use in margarines, shortenings, and cooking and salad oils. Soybean oil generally has a smaller contribution to soybean’s overall value compared to soybean meal, because the oil constitutes just 18 to 19% of the soybean's weight (Figure 4). Nonetheless, soybean oil accounted for approximately 30% of all the vegetable oils consumed globally and was the second largest source of vegetable oil worldwide, slightly behind palm oil at approximately 32% share (Soya and Oilseed Bluebook, 2008).

Figure 4. Oil accounts for approximately 20% of the weight of the soybean seed. Oil is typically produced by crushing the soybean seed followed by extraction of the oil by a process referred to as “solvent extraction.” Source: [https://www.flickr.com/photos/unitedsoybean/10059732523](https://www.flickr.com/photos/unitedsoybean/10059732523)

Soybean meal is used as a supplement in feed rations for livestock. Soybean meal is the most valuable component obtained from processing the soybean, accounting for roughly 50-75% of its overall value (Figure 5). By far, soybean meal is the world's most important protein feed, accounting for nearly 69% of world protein meal supplies (ASA, 2008). Industrial uses of soybean range from a carbon/nitrogen source in the production of yeasts via fermentation to the manufacture of soaps, inks, paints, disinfectants, and biodiesel. Further information on industrial uses of soybean are described by Cahoon (2003) and the American Soybean Association (ASA, 2008).
Figure 5. Soybeans are composed of approximately 40% protein by weight and are one of the most plentiful and low cost sources of protein used in animal feed in the U.S.

Development of Roundup Ready Soybean

The first Roundup Ready soybean event\(^4\) was developed in 1990 by introducing the \(cp4\) \(epsps\) coding sequence shown in Figure 5 below into a maturity group five soybean variety.

DNA Insertion by Particle Bombardment

There are multiple ways to insert DNA into plant cells, including \textit{Agrobacterium}-mediated and particle gun-mediated insertion. Most of the biotechnology crop products on the market today were genetically modified using the \textit{Agrobacterium}-based plant transformation method. However, Roundup Ready soybean was produced through particle gun bombardment, as depicted in Figure 7. The particle bombardment method starts with coating tungsten or gold particles (microprojectiles) with plasmid DNA. The coated particles are coated on a macro-projectile, which is accelerated with air pressure and shot into plant tissue on a Petri plate as shown in Figure 7. A perforated plate is used to stop the macro-projectile, while allowing the microprojectiles to pass through to the cells on the other side. As the microprojectiles enter the cells, the plasmid DNA is released from the particle surface. Some of the DNA will then be incorporated into the chromosomal DNA of the cells. The transformed plant cells are then regenerated into whole plants using tissue culture.

Figure 6. Plasmid used to create Roundup Ready Soybean. A plasmid is composed of genetic elements (DNA) that have been spliced together using genetic engineering techniques. The plasmid used to create Roundup Ready soybean contains: (1) the gene of interest (\(cp4\) \(epsps\)) responsible for producing the CP4 EPSPS protein in the transformed plant (2) a selectable marker (\(nptII\)) needed to select transformed plant cells, (3) promoters and terminators to start and stop expression of the inserted genes (promoters - \(P\)-\(FMV\), \(P\)-\(nptII\); terminator – NOS 3'). Source: Monsanto Company

\(^4\) Each time a new genetically modified plant is created the gene inserts in a unique genomic location. An event is a code name associated with the gene and unique location in the genome.
How does Roundup Ready Soybean work? Tolerance to glyphosate\(^5\), the active ingredient in Roundup agricultural herbicides in Roundup Ready soybean, was achieved through the production of the naturally occurring glyphosate-tolerant CP4 enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein. The EPSPS enzyme is present in both plants and microorganisms. As glyphosate inhibits this enzyme, the plant’s growth slows due to lower amino acid and protein production (Figure 8). Amino acid and protein levels are not affected in mammals, birds, or fish; this is why scientists consider glyphosate to be a low risk to humans and the environment. Production of the glyphosate tolerant CP4 EPSPS protein in plants makes the plants tolerant to Roundup.

\(^{5}\)Glyphosate is a commonly used herbicide (weed killer) found in the Roundup family of herbicides. It is used by farmers and other where weed control is needed.

**Roundup Ready Soybean’s Role in Sustainable Agriculture**

Weeds are a constant challenge for every farmer, and herbicides are the primary and most effective tool for farmers to control weeds and maximize crop yields. It is estimated that the use of herbicides saves over one billion hours of hand labor, contributes to over $15B dollars in increased profit for farmers and saves over $336 million in fuel costs each year, which makes food production more reliable and less expensive for consumers. Using herbicides (rather than other weed control methods like hand weeding and tillage) also reduces labor, increases convenience, conserves soil, and makes food less expensive to produce.

One of the major benefits of herbicide-tolerant crops is their contribution to sustainable agriculture. Sustainable
agriculture is recognized as an important objective by government, industry and farm groups. Those who practice sustainable agriculture believe in efficient production practices, farm profitability, environmental stewardship, and quality of life (See Figure 9). Herbicide-tolerant crops like Roundup Ready soybean support all of these objectives.

An important positive environmental impact attributed to herbicide tolerant crops is that they help farmers practice reduced tillage methods on their farms. Tillage (plowing a field) disturbs the soil and causes erosion, contributing to the pollution of lakes, rivers, and steams, and loss of valuable and nutrient-rich topsoil. Herbicide-tolerant crops allow farmers to use herbicides rather than tillage to manage weeds before and during the growing season.

Our herbicide resources are being challenged by the evolution and spread of herbicide resistance in a growing number of weedy plants. This phenomenon is not new to the use of herbicides associated with biotech crops and impacts all of the herbicides used in agriculture, including glyphosate.

Figure 9. The need to conduct farming operations in a sustainable way is recognized as an important objective by all sectors, including government, industry and farm groups. There are many definitions of sustainable agriculture, but all contain elements that encompass efficient production practices, farm profitability, environmental stewardship and quality of life. Weed management and weed resistance management are important parts of implementing sustainable agricultural operations. Source: Monsanto Company.

Diversified Weed Management
The overreliance on a single herbicide contributes to the development of resistant weeds (Figure 10). One of the strategies to manage the development of herbicide-resistant weeds is called diversified weed management. Diversity can be achieved by using mechanical, cultural, and biological methods, as well as herbicides. Including herbicides with multiple mechanisms of action is considered a good strategy to prevent the development of and control of herbicide-resistant weeds, particularly where herbicides are the sole source of weed control (Figure 11).

Our herbicide resources are being challenged by the evolution and spread of herbicide resistance in a growing number of weedy plants. This phenomenon is not new to the use of herbicides associated with biotech crops and impacts all of the herbicides used in agriculture, including glyphosate.

Figure 10. The progression of weed resistance starts with one resistant plant growing to a population dominated by naturally-occurring biotypes with resistant alleles. Source: Monsanto Company.

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Tillage practices can take several forms including conventional, conservation and reduced tillage methods. Conventional tillage is the most disturbing to the soil resulting in erosion and reducing organic matter in the soil. For more information on tillage see: [http://www.epa.gov/oecaagct/ag101/cropsoil.html](http://www.epa.gov/oecaagct/ag101/cropsoil.html)
Figure 11. Where herbicides are the primary method of weed management, multiple herbicides with overlapping activity and different modes of action applied in mixtures, sequences and/or in rotation are needed to proactively reduce the potential for resistance. Source: Weed Science Society of America
Activities

Activity 1 – Anticipatory Set: Weeds and Planted Crop Competition Simulation

Activity 2 – Growing Roundup Ready Soybeans and Conventional Soybeans

Activity 3 – Quickstrip Test using Whole Seed- Protein detection

Activity 4 – Quikstrip Test using Leaf Tissue- Protein detection

Activity 5 – PCR Test using Soybean Tissue

*Note to Educator: Not required to complete all activities or in order listed. Please determine appropriate activity(s) for your students.
Activity 1 – Anticipatory Set: Weeds and Planted Crop Competition Simulation

Materials for Activity 1

- 3 different colored poker chips (not included)
  - Blue = Water (10-25 chips)
  - Red = Sun (10-25 chips)
  - Black = fertilizer (10-25 chips)
- 3 bags to hold chips (not included)
- Basket (not included)
- 2 colored cards (green and red) (not included)
  - Red = weed
  - Green = planted crop

Introduction:

Farming can be a gamble, but technology can help improve a farmer’s chance of producing a successful crop. Farmers make dozens of decisions each year that can determine the successfulness of the farming business. These decisions may include: type of crop to plant on each field, when to plant, type of seed hybrid to plant, seed source, fertilizer, equipment, herbicides, insecticides, fungicides the type and quantity of fertilizer to apply, type and frequency of herbicide application, at what price point to sell their crop on the global market, etc. All of these decisions impact the success of a farm.

Managing weed growth is a challenge that can have significant economical implications for farmers. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001). Weeds can cause millions of dollars in damage for farmers per growing year. Farmers use a variety of technologies to mitigate yield loss. Some of these technologies include the use of biotech crops (also known as genetically modified or genetically engineered crops).

Discuss with students the requirements that plants need to grow and remain healthy. Possible answers should include: nutrients, water, minerals, sun, air, CO₂, etc. Ask students, “When you are hungry, how do you get the nutrients you need?” Responses from students will likely vary from kitchen to grocery store to restaurant. Remind the students that when plants are hungry or need resources they cannot simply get up and walk to the kitchen, grocery store or restaurant. They are restricted to the resources that fall within their growing space. In this simulation, the students are the plants; some students will represent planted crops while others will represent weeds. Discuss the difference between a desired plant and weeds. Both weeds and crops are plants; weeds are plants that are not desired in a growing area. Corn growing in a soybean field would be considered to be a
weed, because it is not the desired plant for that field.

In this simulation we will demonstrate how devastating weeds can be to a farmer.

Explain to students that they will stand in place for this activity. Their feet will simulate their roots, and they are allowed to move their arms, but not their feet. They must compete to gain the resources (food, light and water) around the area in which they are “planted.”
**Procedure:**

**Round 1**

“Mother Nature” is the educator facilitating the simulation.

Students will be divided into 3 groups: 3 students are needed to represent the resources of rain, sunlight, fertilizer. The remaining students should be divided into two groups with a 3:1 ratio of crops to weeds.

Prepare a representative sample of green and red cards in the 3:1 ratio to account for the numbers of students in your class. Students should grab a colored card (green = planted crop, red = weed) out of a basket. This will help to divide them in 75% plants 25% weeds at random.

Students with green cards should align themselves into rows to simulate a field of a planted crop. Students should be far enough apart that they cannot touch when arms are extended.

Students with red cards will plant themselves anywhere in the field, randomly dispersed among the crop plants. Figure 2. 25% weed simulation.
Mother Nature coordinates the simulation by instructing the three students selected earlier to decide when and where the resources of rain, fertilizer and sunlight will be available to plants. The students representing fertilizer, water and sunlight can sprinkle their "chips" around the feet of the students serving as crops and weeds to distribute the resources to the all plants.

Blue chips represent water, red chips represent the sun, and black chips represent fertilizer that will be dropped near plants by the three students selected by the instructor.
Mother Nature instructs the elements to align themselves randomly in the field.

They can move around as they please during the simulation.

For example: one section of the field may receive a lot of sun but no water.

Then Mother Nature instructs the element students to gently sprinkle poker chips toward the students when instructed to do so.

For example: “Water, you may make it rain a lot.” [wait a couple seconds] “Okay, water, stop raining.” [water stops tossing poker chips]
The students- both planted crop and weeds scramble to pick up as many poker chips as they can without moving their feet (roots).

The students who have exactly 3 of each color may stay standing and go to harvest.

*note: too much rain, fertilizer or sun can also damage plants.
The rest of the plants did not have the resources needed to grow and will sit down. These plants have perished due to a lack of resources.

Repeat the simulation for two more rounds with the following percentages of planted crop students to weed students.

**Round 2**
Divide students into 2 groups 50% weeds 50% planted crops

**Round 3**
Divide students into 2 groups 75% weeds 25% planted crops
**Conclusion:**

Students will start to see the devastating effects of weeds in the field. The more weeds in the field, the more competition that the planted crops have to compete for resources.

**Knowledge Check Questions to ask during the game:**

- What resources/elements do plants need to survive?
- What challenges do farmers face when planting crops?
- How would a farmer describe a weed?
- Why are weeds a problem for farmers?
- What are the different technologies farmers can use to protect against weeds?
- What are the benefits and risks of these technologies?
- If you were a farmer, what technology would you invest in and why?
Activity 2- Growing Roundup Ready Soybeans and Conventional Soybeans

Materials for Activity 2

- Seeds –
  - Conventional soybean (variety A3525)
  - Roundup Ready (MON-04032-6 in an A3525 background)
  - Optional: Weed Seeds (not included), Williams 82 variety soybean (conventional soybean)
- Soil media (not included)
- Pots / trays (not included)
- Water (not included)
- Roundup Herbicide\(^7\) (not included)

**Note:** Use product according to directions on the label and choose a product that lists only glyphosate as the active ingredient. Label instructions and appropriate precautions must be followed.

\(^7\) Glyphosate is the active ingredient in Roundup Herbicide. Be sure to review the product label and select a Roundup herbicide that lists only glyphosate as the active ingredient.
Introduction:
In this activity, students will compare the growth of Roundup Ready and conventional soybeans by planting both types of soybeans. Additionally, students will observe the effects of applying Roundup herbicide to Roundup Ready soybeans and conventional soybeans. Common weed seeds can be planted at the same time as the soybean seed to simulate weeds in a field. However, this is not necessary, because students will see the impact of Roundup herbicide either way. Roundup herbicide will be applied at an early growth stage once the soybean and weed seeds (if planted) have sprouted. Students will observe the effect of the herbicide on non tolerant soybean and weed plants and discuss impacts to farming and food production. Optional activities include growing additional plants to later growth stages and comparing the later stage growth patterns of Roundup Ready soybean to conventional soybean.

The Roundup Ready and conventional A3525 seed provided in this kit are derived from a maturity group 3.5 soybean (See Soybean Variety Development, pg 7). The conventional A3525 soybean and Roundup Ready soybean have been carefully prepared and are closely matched genetically. The key difference between the Roundup Ready and the conventional seed is that the Roundup Ready soybean contains the cp4 epsps gene. The impact of this gene on the plant is to make the plant tolerant to Roundup herbicide. Research conducted by Monsanto and reviewed by global regulatory agencies confirms that the gene results in no other phenotypic changes to the plant. For example, the research showed that the presence of the transgene among the thousands of other genes in the soybean plant did not affect the nutrient composition or agronomical characteristics of the soybean. Apart from the introduced gene and the glyphosate-tolerance phenotype it confers, the biotech soybean plant is no different from the conventional soybean plant.

Modern plant breeding is based on the gene as the unit of heredity and how genes behave. Breeders make controlled cross-pollinations between plants with different combinations of genes and then use keen observations and decision making skills to select for improvements in the plants they are studying. Through this process the breeders are recombining existing genes into new combinations and selecting for the desired characteristics, which often include yield, nutrition, abiotic stresses (e.g. drought) agronomic performance, disease and insect resistance and many others. In the case of soybeans, it is estimated that the genome contains 1.1 gigabases of DNA with 46,430 protein-coding genes. Note that most genes have one or more forms (alleles). Thus, there are many combinations of genes.

8 Characteristics of plants that can be observed or measured are referred to as the “phenotype of the plant.” Characteristics include germination, vigor, leaf type, growth pattern, time to flowering, flower color, fruit or seed composition/quality and yield.

9 See USDA Petition for Roundup Ready Soybean at the following link for more information: http://www.aphis.usda.gov/brs/aphisdocs/93_25801p.pdf
and alleles that can be assessed by breeders to improve soybeans. Some desired characteristics do not occur in the gene pool of a crop plant and cannot be obtained by cross-pollination and selection. However, biotechnology advancements have allowed for enhancements of plants with one or more genes using transformation that do not occur in the gene pool of the crop of interest (in this case soybean). Although this technique is how Roundup Ready soybean was developed, it is still necessary for plant breeders to continue to select for improved yield and other characteristics to continually improve soybean productivity. The addition of the Roundup Ready trait is one gene that provides tolerance to glyphosate (one trait), but there are thousands of other genes in soybean that are involved in plant growth and development.

One characteristic that breeders may select for is flower color. Soybean flowers can have white or purple petals. The $W1$ allele (one of two forms of the gene) of soybean is associated with a purple flower color and purple hypocotyl (region between the roots and shoots of young seedlings). The recessive homozygote ($w1 w1$) has white flowers and green hypocotyls, while the heterozygote ($W1 w1$) and dominant homozygote ($W1 W1$) has purple flowers and purple hypocotyls. Thus, there is a single gene that influences the phenotype of the plant that can be observed. Students who plant the Williams 82 soybean seed and the A3525 soybean will notice these phenotypic differences. These differences are attributed to the existing genes in soybean and are one example of the effect of an allele of the same gene on the phenotype of the plant.
Procedure for plant growth and demonstration of Roundup’s effect on tolerant and non-tolerant plants:

Planting seeds: Procedure from start to end should take approximately 30 days.

1. Gather Roundup Ready soybean seeds, conventional soybean seeds, planting trays, soil, labels, and permanent marker.
2. Label one tray as RR for Roundup Ready and one tray as CONV for conventional soybeans. Place the date of planting on both labels and place one label in each tray.
3. Place tray with holes into tray without holes. Fill trays with soil media, level soil throughout the tray, and saturate with water. Allow excess water to drain. Dump extra water from bottom tray.
4. Plant seeds with uniform distribution approximately 2 inches apart. Use a pencil to press a hole into the moist soil 1/2 inch deep with 2 inch spacing, creating a grid. Drop a soybean seed into each hole and sprinkle soil on top of all seeds. Place trays in a location with bright light for at least 8 hours and warm temperatures of 75-85°F. For best results, add water to the bottom tray.
5. Check plants daily for watering and growth. Water trays only if soil is dry, avoid overwatering, but be sure to keep plants from wilting.
6. Create a table with column headings of Date, Temperature, Plant Growth, and Observations.
7. Students should note changes in growth, leaf color, sketches of plants, temperature, moisture levels, etc. in their notebook or data sheets.
8. Record plant growth for 20 days or until most of the soybean plants have four true leaves. The first two leaflike structures that emerge from the soil are cotyledons (See Figure A2.2).
9. Latex gloves should be utilized while handling any chemicals, such as Roundup herbicide.
10. Spray a fine mist of Roundup solution on the leaves of the soybeans to just barely moisten the leaves. Record observations on plant growth for 5 days on both trays. Use Roundup as indicated in the label.
Figure A2.1. Demonstration of the Roundup Ready weed control system. Roundup Ready and control A3525 soybeans are planted in separate trays. Optional to plant weed seeds along with the soybean. Plants are allowed to grow and are treated with Roundup herbicide around the V2 growth stage (See soybean growth stages, Figure A2.3). Source: Monsanto Company
Phenotypic characterization of Roundup Ready, conventional and Williams 82 soybean

1. Plant Roundup Ready and control seeds in separate 6-inch pots. Plant three seeds per pot and thin to one seedling per pot after plants produce their first true leaves. Continue to grow plants as described in A2.2. Be sure to plant a minimum of three replicates (i.e. three pots) for each seed type, and calculate a mean for all comparisons.

2. Compare the growth patterns of Roundup Ready versus the two control groups at various growth stages.

3. Suggested comparisons (See Figure A2.3 for growth stages):
   a. Time to emergence/number emerged
   b. Cotyledon color
   c. Leaf shape
   d. Plant height (measured at the soil to uppermost growing leaf tip)
   e. Plant vigor
   f. Biomass (cut plant at the base and weigh plant or weigh entire plant after removing from soil and washing roots)
   g. Time to flower\(^{10}\)

---

\(^{10}\) Flowering for soybean can be difficult. To set flowers indoors, you will need to have control over the light and dark cycles. A 10 hr day and 14 hr night starting no earlier than V6 stage which could be accomplished in a class room by placing large boxes over plants for night time cycle.
Figure A2.2. Newly emerged soybean showing cotyledons, leaves and hypocotyls. Note differences in hypocotyl color associated with the Williams 82 variety of soybean (green) versus soybean of the A3525 variety. Source: Monsanto Company

Figure A2.3. Soybean growth and development stages. Source: http://weeds.cropsci.illinois.edu/extension/Other/POCKETcrop.pdf
**Student Observations:**

Have students sketch their experimental setup on day 1

Have each student select one plant in the tray to record measurements, observations and sketches in their notebook.

When were you able to determine a difference in the appearance of the RR vs Conventional soybeans?

How many days passed between the Roundup application and the visibility of damage to the susceptible plants?

**Conclusions (Activity 2A)**

The genetically engineered Roundup Ready soybeans are tolerant to Roundup herbicide while conventional soybeans fail to survive. Therefore, RR soybeans provide farmers with a safe, convenient way to eliminate weeds and produce more soybeans.

**Conclusions (Activity 2B)**

(1) Other than the intended change (e.g. tolerance to Roundup), the *cp4 epsps* gene does not change other phenotypic characteristics of soybean (e.g. cotyledon color, plant height, biomass, etc.), (2) the effect of different endogenous soybean genes can be seen through cotyledon and flower color comparisons of A3525 and Williams 82.
Activity 3 - Quickstrip Test using Whole Seed- Protein Detection

Materials for Activity 3

- Soybean Seeds –
  - Conventional soybean
  - Roundup Ready (MON-04032-6)
- Microfuge tubes, 1.5 – 2.0 mL size (2 / student; not included)
  - Fisher 05-402-25 or similar
- Transfer pipette or a single channel mechanical pipette and tips (capable of measuring ~0.5mL of PBS) (1/student) (not included)
  - Transfer pipette VWR 16001-188 or similar
- Lateral flow strips (Romer AgraStrip® RUR-HS Item No. 7800013 – 50 strips per kit) (not included)
- Timer (not included)
- Marker (1/ 4 students) (not included)
- Paper and pen (1/student) (not included)
- Hammer/ mallet (multiple- students can take turns) (not included)
- Tweezers/small forceps (1/ student) (not included)
- Gloves (to help control cross-contamination) (not included)
- 50-70% Ethanol (to clean tweezers or forceps between sample preps) (not included)
- Paper towels (to wipe tweezers or forceps after Ethanol cleaning) (not included)
- 1% PBS buffer (0.5mL/ student) (not included)
- Microfuge tube rack (1/student) (not included)
- Weight boats (4/ student ) (not included)

Introduction:

Lateral flow immunoassay. This technique is commonly used to detect the presence of proteins produced by genetically modified plants. This is also the same technology used in for in-home diagnostic tests like home pregnancy tests. The biotech industry uses this technology for selection of traits in plant breeding, for quality control and purity testing of seed batches or for plants used to produce seed and to support GMO labeling. The technique employs the use of protein specific antibodies that bind to a protein (antigen) that is produced by the GMO plant. This same protein is not present in the conventional plant and is produced as a result of the inserted DNA. There are multiple antigens on the surface of each protein, so multiple antibodies can bind to the protein as illustrated in Figure A3.1. One of the antibodies is labeled (the detection antibody), and one of the antibodies is anchored to the surface of the membrane and “captures” the labeled antigen-antibody complex. This antibody-antigen-antibody sandwich form a pink line on the membrane when the protein is present. A similar control line forms for the antibody-antibody sandwich, indicating that the test system has functioned properly.
Figure A3.1. The lateral flow immunoassay occurs on a nitrocellulose membrane. Proteins are extracted from a plant tissue sample and the sample (plant extract) is applied to the sample pad. Proteins in the sample are absorbed by the “sample pad” and migrate into the membrane and flow towards the “wicking pad.” As the sample flows through the membrane, labeled antibodies are solubilized from the “antibody conjugate pad” and migrate along with the sample. The labeled antibodies react (bind) to specific proteins in the plant extract. The “test line” contains antibodies that also react with the protein. Capture antibodies are immobilized along the test line and when the protein-labeled antibody complex reaches this point, a “sandwich” is formed creating a visible line as the conjugate antibody – protein – capture antibody complex piles up. A second “control line” forms further up the membrane where a capture anti-conjugate antibody is immobilized along the control line. The antibody conjugate piles up at this line indicating that the test strip is functioning. In the absence of specific plant protein, only the secondary line is visible. From Grothaus et al, 2006. Source: Monsanto Company
Procedure:

Whole Soy Seed:

1. Label microfuge tubes for identification of the seed that will be tested
   a. Label 1 microfuge tube “RR soy” Label the other microfuge tube “conv soy”
   b. Label one plastic bag “RR soybean.” Label the other plastic bag “conventional soybean.”
2. Break a soy seed
   a. Place 1 RR soybean seed between 2 small weigh boats and tap with a hammer, as long as the seed is broken into 2-3 pieces there will be enough surface area exposed for extraction. Crushing the seed can cause issues recovering all the pieces for extraction and may create a cross contamination of the testing area.
3. Remove the top weigh boat and place the seed pieces into a microfuge tube. Bending the boat to funnel the seed into the tube works most of the time. If the seed is stuck to the boat, use tweezers to gently release it.
4. Repeat seed crushing method for conventional soybean with 2 unused weight boats
5. Add ~0.5 ml of 1% PBS into tube with the seed.
6. Close the cap and shake vigorously for 20-30 seconds.
7. Let the tube stand for 3-5 min before testing.

Use of AgriStrip RUR-HS test strips:

a. Remove strips to be used, reseal canister immediately.
b. Place 1 strip per microfuge tube, with the arrow pointing down.
c. Incubate LFS at room temp for 5 min
   a. You may see positive results earlier than 5 min, however full incubation time will allow for the negative control to fully develop.
Interpreting the Results:

1. A single control line should develop, if it does not, dispose of strip and retest sample with a new strip.
2. If the sample contains CP4 EPSPS protein, a second line will develop between the control line and the tape with the arrow on it. If the sample does not contain the CP4 EPSPS protein, a second line will NOT be present on the test strip.

Troubleshooting/Common Errors:

- Cross-contamination
  - Messy testing area
    - Wiping down a work area frequently or using a disposable cover (paper towel) on the work area that can be changed between sample types can reduce this type of contamination
  - Contaminated tools
    - Using ethanol to clean tools (such as tweezers) between samples can reduce this type of contamination
  - Messy hands
    - Wearing gloves and changing them between samples can help reduce this type of cross-contamination

- Shortening or lengthening the incubation time
  - Strips should be run for at least 5 min for negative sample to fully develop and be declared negative
  - Running the strips too long can cause discoloration or fading of the results due to the excess buffer pulling through

- Too little or too much water
o Too little water can prevent the extraction from running past the reaction/test window
o Too much water can flood the strip causing it to run too fast or to dilute. Extract that is higher than the top of the bottom filter pad is an indication that too much water was added.

**Recording the Results:**

1. Find sample number on tube and record on chart
2. Record the sample identity and type (leaf or seed)
3. Record positive (+) or negative (0) on chart

**Example Results chart:**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Sample identity</th>
<th>Sample type</th>
<th>Control line</th>
<th>Test line</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Conv</td>
<td>Leaf</td>
<td>+</td>
<td>0</td>
<td>No CP4 EPSPS detected</td>
</tr>
<tr>
<td>2</td>
<td>Conv</td>
<td>Seed</td>
<td>0</td>
<td>0</td>
<td>Invalid test strip</td>
</tr>
<tr>
<td>3</td>
<td>RR</td>
<td>Leaf</td>
<td>+</td>
<td>+</td>
<td>Positive for CP4 EPSPS</td>
</tr>
<tr>
<td>4</td>
<td>RR</td>
<td>Seed</td>
<td>+</td>
<td>+</td>
<td>Positive for CP4 EPSPS</td>
</tr>
</tbody>
</table>

**Conclusion:**

Presence of CP4 EPSPS protein that confers glyphosate tolerance was identified in Roundup Ready soybeans while it was absent from conventional soybeans. This explains the tolerance that RR soybeans have to the Roundup herbicide

**Comprehension Questions:**

What is a lateral flow strip and how does it work?

What is a control and why are controls necessary in research? List two controls that are found in this activity.
Activity 4 – Quikstrip Test using Leaf Tissue- Protein Detection

Materials for Activity 4 – Quikstrip Test using Leaf Tissue- Protein detection

- Seeds
  - Conventional soybean
  - Roundup Ready (MON 04032)
- Soil media (not included)
- Pots / trays (not included)
- Water (not included)
- Microfuge tubes, 1.5-2.0 mL size (2 / student) (not included)
  - Fisher 05-402-25 or similar
- Tap water (not included)
- Container for tap water (50 mL conical work well) (not included)
- Transfer pipette or a single channel mechanical pipette and tips (capable of measuring ~0.5mL of PBS) (1/student) (not included)
  - Transfer pipette VWR 16001-188 or similar
- Lateral flow strips (Romer AgraStrip® RUR-HS Item No. 7800013 – 50 strips per kit) (not included)
- Timer (not included)
- Marker (1/ 4 students) (not included)
- Paper and pen (1/student) (not included)
- Gloves (to help control cross-contamination) (not included)
- Paper towels (to wipe tweezers or forceps after Ethanol cleaning) (not included)
- 1% PBS buffer (0.5 mL/ student) (not included)
- Microfuge tube rack (1/student) (not included)
- Micro-pestle (2/ student) (not included)

Introduction:
This technique is commonly used to detect the presence of proteins produced by genetically modified plants. This is also the same technology used in for in-home diagnostic tests like home pregnancy tests. The biotech industry uses this technology for selection of traits in plant breeding, for quality control and purity testing of seed batches or for plants used to produce seed and to support GMO labeling. The technique employs the use of protein specific antibodies that bind to a protein (antigen) that is produced by the GMO plant. This same protein is not present in the conventional plant and is produced as a result of the inserted DNA. There are multiple antigens on the surface of each protein, so multiple antibodies can bind to the protein as illustrated in Figure A4.1. One of the antibodies is labeled (the detection antibody) and one of the antibodies is anchored to the surface of the membrane and “captures” the labeled antigen-antibody complex. This “antibody-antigen-antibody sandwich form a pink line on the membrane when the protein is present. A similar control line forms for the antibody-antibody sandwich indicating that the test system has functioned properly.
Figure A4.1. The lateral flow immunoassay occurs on a nitrocellulose membrane. Proteins are extracted from a plant tissue sample and the sample (plant extract) is applied to the sample pad. Proteins in the sample are absorbed by the “sample pad” and migrate into the membrane and flow towards the “wicking pad.” As the sample flows through the membrane, labeled antibodies are solubilized from the “antibody conjugate pad” and migrate along with the sample. The labeled antibodies react (bind) to specific proteins in the plant extract. The “test line” contains antibodies that also react with the protein. Capture antibodies are immobilized along the test line and when the protein-labeled antibody complex reaches this point, a “sandwich” is formed creating a visible line as the conjugate antibody – protein – capture antibody complex piles up. A second “control line” forms further up the membrane where a capture anti-conjugate antibody is immobilized along the control line. The antibody conjugate piles up at this line indicating that the test strip is functioning. In the absence of specific plant protein, only the secondary line is visible.
Procedure:

Soybean leaf tissue:

Use a leaf at the V2 through V4 stage (See Figure 1, Soybean Growth and Development Stages, pg 7)

1. Label microfuge tubes for identification of the plant that will be tested
   a. “RR soy” Label the other microfuge tube “non RR soy”
2. Take a 4 disk leaf punch using the microfuge tube
   a. Remove 1 leaf from the plant, fold the leaf in half and then in half again
   b. Place the folded leaf between cap and body of tube. Close the lid of the tube, tear away extra leaf from around the outside of the closed tube and allow the leaf punches to fall into tube.

3. Use a coffee stirrer or other device to push leaf punches into bottom of tube.
   a. Warning: DO NOT grind the tissue yet! Over extraction can cause chlorophyll to collect in the test band line of the strip and make it difficult to interpret the results.
4. Use Pipette to add ~0.5mL of 1% PBS into tube containing leaf tissue.
   a. pipette 0.5mL of PBS and add solution to “RR soy” microfuge tube
   b. pipette 0.5mL of PBS and add solution to “conv” microfuge tube
5. Macerate the leaf (smash up/grind).
   a. crush leaf tissue with micropestle until leaf broken into pieces
   b. This doesn’t take much once you see some green color to the water the sample has extracted.
6. Close cap and shake vigorously for 20-30 seconds to mix.
7. Proceed to testing.
8. Place microfuge tubes into rack.
Use of AgriStrip RUR-HS test strips:

1. Remove strips to be used, reseal canister immediately.
2. Place 1 strip per microfuge tube, with the arrow pointing down
3. Incubate LFS at room temp for 5 min
   a. You may see positive results earlier than 5 min, however full incubation time will allow for the negative control to fully develop.

Interpreting the Results:

1. A single control line should develop, if it does not, dispose of strip and retest sample with a new strip (figure b.1).
2. If the sample contains CP4 EPSPS protein, a second line will develop between the control line and the tape with the arrow on it. If the sample does not contain the CP4 EPSPS protein, a second line will NOT be present on the test strip.

Troubleshooting/Common Errors:

- Over-extraction of the samples
- See below for more detail on over extracting leaf samples

If leaf samples are over-extracted (i.e. due to over-maceration) they will show an intense green color. This can result in chlorophyll deposits on the positive test line.
Recording the Results:

1. Find sample number on tube and record on chart
2. Record the sample identity and type (leaf or seed)
3. Record positive (+) or negative (0) on chart

Example Results chart:

<table>
<thead>
<tr>
<th>Sample number</th>
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<th>Sample type</th>
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<td>2</td>
<td>Conv</td>
<td>Seed</td>
<td>0</td>
<td>0</td>
<td>Invalid test strip</td>
</tr>
<tr>
<td>3</td>
<td>RR</td>
<td>Leaf</td>
<td>+</td>
<td>+</td>
<td>Positive for CP4 EPSPS</td>
</tr>
<tr>
<td>4</td>
<td>RR</td>
<td>Seed</td>
<td>+</td>
<td>+</td>
<td>Positive for CP4 EPSPS</td>
</tr>
</tbody>
</table>
Activity 5 – PCR Test using Leaf Tissue

Materials for Activity 5

- Conventional soybean
- Roundup Ready soybean (MON-04032-6)
- Seed germination towels (paper towels)
- Microfuge tubes, 1.5-2.0 mL size (2 / student) (not included)
  - Fisher 05-402-25 or similar
- Sterile Gloves
- Chlorine bleach solution (5% bleach in distilled water)
- Extraction buffer (200 mM Tris HCl at pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS)
- Microcentrifuge capable of generating 12,000 RPM
- 37°C incubator
- Thermocycler
- Gel electrophoresis equipment

Introduction

This technique is commonly used to detect the presence of genetic elements inserted into the genome of genetically modified plants. This technology has been broadly applied across many areas including medical, infectious disease, forensics and research applications. The agricultural biotechnology industry uses this technology for selection of traits in plant breeding, for quality control and purity testing of seed batches or for plants used to produce seed and to characterize the genetic inserts and to support GMO labeling.

E-learning: Tutorial on Polymerase Chain Reaction (PCR)
Figure A5.1. Polymerase Chain Reaction (PCR). Step 1 Denaturation: double stranded DNA is denatured using high temperatures, Step 2 Annealing: primers anneal to homologous sequences in single stranded DNA, Step 3 Elongation: DNA polymerase elongate the primers to synthesize new double stranded DNA. Steps 1 through 3 are repeated multiple times to generate large amounts of specific DNA. Source: Monsanto Company

PCR procedure for Roundup Ready soybean

Seed Sterilization and Germination

1. Sterilize each soybean seed in a labeled sterile 1.5 ml tube.
2. Add soybean seed to 950 µl of 5% bleach let stand for 10 min.
3. Decant bleach and rinse seed 6 times with 950 µl of sterile water.
4. Wet a clean absorbent paper towel in sterile water so that the towel is wet (not soaked but more than just damp).
5. Fold seeds in paper towel, keeping like seeds together.
6. Spray the outside of the paper towel with 5% bleach to keep mold from growing.
7. Place the wet paper towel into an unused clean Ziploc bag. Do not seal the bag completely.
8. Place bag into a 32°C incubator and incubate 1-2 days until germination. You will clip a small bit of the root and allow the seedling to keep growing to test for resistance to glyphosate.
Steps | Function
--- | ---
Tissue (leaf) | Sample
Physically macerate tissue | Cell Lysis
Add detergent (SDS) | Removes lipid membranes / denature proteins
Add alcohol (isopropyl) | Precipitate DNA
Add alcohol (70% Ethanol) | Further remove co-contaminants like salt
Add water or buffer (TE) | Dissolve genomic DNA
DNA Extraction Protocol (Sambrook and Russell, 2001a)

1. Rinse the germinating seedling then using your gloved finger, break off 1-2 cm of an emerging root and add it into a clean 1.5 ml tube. Put the germinating seedling back into the wet paper towel to continue germinating.
2. Macerate tissue using a pestle. Be careful to not flick your tissue out of the tube with the homogenization. You will still have some solid pieces, but you should have a slurry.
3. Add 400 µL of extraction buffer (200 mM Tris HCl at pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS).
4. Vortex for 5 seconds.
5. Centrifuge the extract at 13,000 rpm for 3 minute.
6. While centrifuging, add 300µL of isopropyl alcohol to a fresh Eppendorf tube.
7. Add 300µL of the supernatant into the isopropyl alcohol tube, take care to not bring any solid material, you may need to add less than 300µL.
8. Leave the mixture at room temperature for 2 minutes.
9. Centrifuge at 13,000 rpm for 5 minutes.
10. Quickly pour off the liquid, making sure not to dislodge the pellet.
11. Close tube, centrifuge for 10 sec.
12. Remove remaining liquid with Eppendorf pipette.
13. Add 1 mL of 70%-75% ethanol to the Eppendorf tube.
14. Centrifuge at 13,000 rpm for 2 minutes.
15. Quickly pour off the ethanol making sure not to dislodge the pellet.
17. Remove remaining liquid with Eppendorf pipette, and leave the Eppendorf tube open for 15-20 minutes at 37°C. Your sample should be dry and should not smell of ethanol.
18. Add 100µL sterile water to the Eppendorf tube to dissolve the pellet. You may need to pipette the liquid up and down vigorously several times to resuspend the pellet. It is highly likely that much of the pellet will not dissolve. If everything dissolved then the DNA should be dissolved in this water and can now be used for PCR amplification. Be sure your tube is labeled. If the pellet is not completely dissolved, proceed to #19.
19. If there is material that did not dissolve, then centrifuge at 13,000 rpm for 3 minutes to pellet the debris that did not dissolve in water and pipette the liquid into a clean sterile tube. The DNA should be dissolved in this water and can now be used for PCR amplification. Be sure your tube is labeled.
PCR Protocol (Sambrook and Russell, 2001b)

This protocol is designed to amplify a 275-bp PCR product that spans the 5’ insert-to-plant junction in soybean 40-3-2. The positive control assay is a soybean endogenous lectin (lec) target and will amplify a PCR product of 400 bp.

1. 40-3-2 PCR assay (event PCR assay)
   a. 20 µL Qiagen Multiplex PCR Mastermix 2x
   b. 0.8 µL of each of 0.2 µM primer (forward and reverse)
   c. 1 µL DNA
   d. 17.4 µl water

2. lec4 PCR assay (positive control PCR assay)
   a. 20 µL Qiagen Multiplex PCR Mastermix 2x
   b. 0.8 µL of each of 0.2 µM primer (forward and reverse)
   c. 1 µL DNA
   d. 17.4 µl water

3. Mix the reaction tubes by tapping on the PCR tube. Your total volume will be 40 µL.
4. Centrifuge briefly to pool the PCR ingredients if necessary.
5. Place the prepared PCR tube into the PCR machine* and run using the following sequence:
   1. 95º C for 10 minutes
   2. 94º C for 15 seconds
   3. 62º C for 30 seconds
   4. 72º C for 30 seconds
   5. Repeat steps 2 – 4 for an additional 44 times (45 cycles total)
   6. 72º C for 5 minutes
   7. Hold at 10º C (samples can be removed from PCR machine and stored in 4°C till use)

*Run the PCR in an Eppendorf Mastercycler Gradient thermal cycler, or equivalent, using the following cycling parameters. Note: Different thermal cyclers might need ramp rate adjustment to Eppendorf default ramp rate (3°C/second).

The following primers are used in this procedure (sequence 5’ to 3’):

- 40-3-2 primer 1: TTT GGG ACC ACT GTC GGC AGA GGC ATC TT
- 40-3-2 primer 2: GAT TTG AAT TCA GAA CCT TGT GCA
- lec primer 1: GTT TGA CAC TTT CCG GAA CTC TTG
- lec primer 2: CTG TCA CAT TTA GAT GGC CTC ATG
## PCR reaction set up

<table>
<thead>
<tr>
<th>Reagent</th>
<th>One Reaction (μl)</th>
<th>Example: Volume added for 10 reactions (μl)</th>
<th>Volume added for ___ reaction (μl)</th>
<th>Check if added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen Multiplex PCR Mastermix 2x</td>
<td>20</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 μM primer (Forward)</td>
<td>0.8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 μM primer (Reverse)</td>
<td>0.8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>17.4</td>
<td>174</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Sample loaded in gel

<table>
<thead>
<tr>
<th>Well</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>


Agarose Gel Electrophoresis

![Agarose Gel Electrophoresis Diagram]

**Figure A5.2. Agarose gel electrophoresis**, Step 1. Prepare appropriate size and percentage agarose gel, Steps 2 and 3. Load DNA samples into all appropriate wells, Step 4. Attach negative electrode to top (side where samples were loaded) of agarose gel and the positive electrode to the bottom (side opposite where samples were loaded), Steps 5 and 6. Run current across agarose gel and monitor migration of DNA. Stop current prior to samples running out of the bottom of the agarose gel. Source: Monsanto Company

**Gel Electrophoresis Protocol (Sambrook and Russell, 2001a)**

1. Load 10 µL of molecular weight marker into one of the electrophoresis gel wells.
2. Load 10 µL of the sample into another well of the electrophoresis gel.
3. Repeat step 2 (using sequential empty wells) until all PCR samples have been loaded into the gel.
4. Run the electrophoresis between 100 and 125 V.
5. Stain gel with Ethidium Bromide.

E-learning: [Tutorial on agarose gel electrophoresis](#)
**Expected Results**

1. The Positive Control Sample must produce the expected bands for the lec-specific (400 bp) and the 40-3-2-specific (275 bp) PCRs
2. The Negative Control Sample must produce the expected band for the lec-specific (400 bp) PCR and must not produce the expected band for the 40-3-2 PCR
3. The no template control must not produce the expected bands for the lec or 40-3-2 PCR

**Questions:**

Which samples are GM and which samples are not? Is this what you expected? Why or why not?

Once your seeds have grown you can transplant into soil, after 1 or 2 weeks spray the seeds with Roundup to see if the plant is Roundup resistance.

What other GMO crops are commercially available? What kinds of GM Soybean or other crop plants are being currently researched?

What needs to be done before a crop plant can move from research to commercial availability?
Literature Cited


