Dolan DNA Learning Center
Cold Spring Harbor Laboratory

Detecting Genetically Modified Foods by PCR

Funded by NSF

INTRODUCTION

Genetic engineering is responsible for the so-called "second green revolution." Genes that encode herbicide resistance, insect resistance, draught tolerance, frost tolerance, and other traits have been added to many plants of commercial importance. In 2003, 167 million acres of farmland worldwide were planted in genetically modified (GM) crops – equal to one fourth of total land under cultivation. The most widely planted GM crops are soybeans, corn, cotton, canola, and papaya.

Two important transgenes have been widely introduced into crop plants. The *Bt* gene, from *Bacillus thuringiensis*, produces a toxin that protects against caterpillars, reducing applications of insecticides and increasing yields. The glyphosate resistance gene protects food plants against the broad-spectrum herbicide Roundup®, which efficiently kills invasive weeds in the field. The major advantages of the "Roundup Ready®" system include better weed control, reduction of crop injury, higher yield, and lower environmental impact than traditional herbicide systems.

Most Americans would probably be surprised to learn that more than 60% of fresh vegetables and processed foods sold in supermarkets today are genetically modified by gene transfer. In 2004, approximately 85% of soy and 45% of corn grown in the U.S. were grown from Roundup Ready® seed.

This laboratory uses a rapid method to isolate DNA from plant tissue and food products. Then polymerase chain reaction (PCR) is used to assay for evidence of the 35S promoter that drives expression of many transgenes. Herbicide resistance correlates with an insertion allele that is readily separated from the wild-type allele by electrophoresis on an agarose mini-gel. Amplification of tubulin, a gene found in all plants, provides evidence of DNA in the preparation, while tissue from wild-type and Roundup Ready® soy plants are negative and positive controls for the transferred gene (transgene). Since soy and corn are ingredients in many processed foods, it is not difficult to detect 35S transgene in a variety of food products.

Castle, L.A., Siehl, D.L., Gorton, R., Patten, P.A., Chen, Y.H., Bertain, S., Cho, H.J., Wong, N.D., Liu, D., Lassner, M.W. (2004). Discovery and Directed Evolution of a Glyphosate Tolerance Gene. *Science* **304**: 1151-1154.

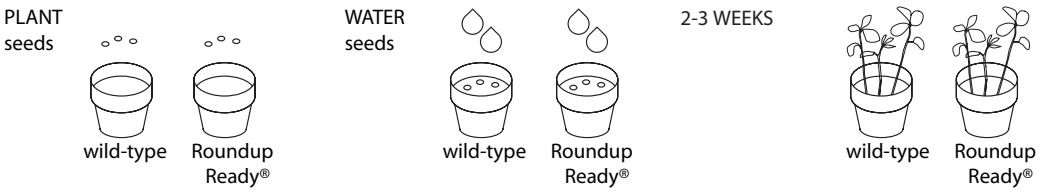
Edwards, K., Johnstone, C. and Thompson, C. (1991). A Simple and Rapid Method for the Preparation of Plant Genomic DNA for PCR Analysis. *Nucleic Acids Res.***19**: 1349.

Stalker, D.M., McBride, K.E., Maiyj, L.D. (1988). Herbicide Resistance in Transgenic Plants Expressing a Bacterial Detoxification Gene. *Science* **242**: 419-423.

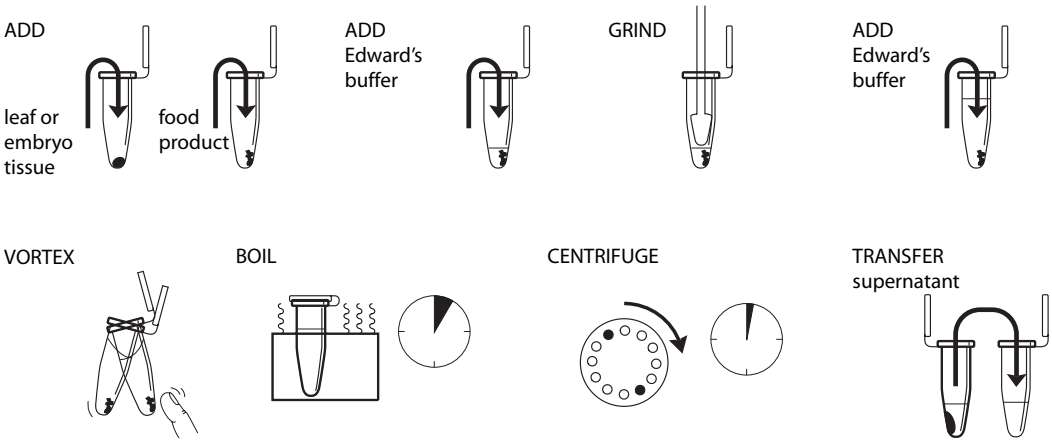
Vollenhofer, S., Burg, K., Schmidt, J., Kroath, H. (1999). Genetically Modified Organisms in Food Screening and Specific Detection by Polymerase Chain Reaction. *J. Agric. Food Chem.* **47**: 5038-5043

LAB FLOW

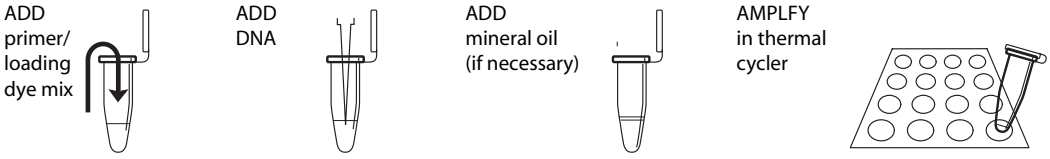
I. PLANT SOYBEAN SEED



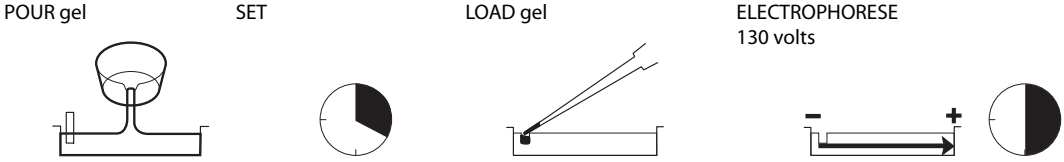
II. ISOLATE DNA FROM SOY AND FOOD PRODUCTS



III. AMPLIFY DNA BY PCR



IV: ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

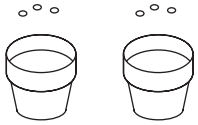


METHODS

I. PLANT SOYBEAN SEED

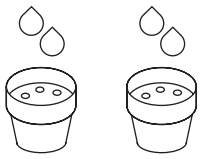
To extract DNA from leaf tissue, you must plant the soybean seeds 2-3 weeks prior to DNA isolation and PCR. Alternately, to extract DNA from embryos, seeds must be soaked in water for at least 30 minutes prior to DNA isolation and PCR.

Reagents	Supplies and Equipment
Wild-type and Roundup Ready® soybean seeds	Planting pot or flat Potting soil



For best results, use a potting soil formulated specifically for soybean

1. Fill the planting pots or flat evenly with potting soil, but do not pack the soil tightly.
2. Label half of the pots "Roundup Ready®," and half of the pots "Wild-type."
3. Plant only 3 of the appropriate seeds per pot, or one per flat cell, to allow optimal growth and easy observation.
4. Use your finger to make a 0.5 inch depression. Add a seed, cover with soil, and lightly tamp.
5. Water the plants from above to prevent the soil from drying out. Drain off excess water, and do not allow the pot or flat to sit in water.
6. Grow the plants close to a sunny window at room temperature or slightly warmer. A growth light may be used.
7. Harvest plant tissue for PCR as soon as the first true leaves become visible. These will follow the cotyledons, or seed leaves. This should be about 2 weeks after planting, depending on light and temperature conditions.
8. Allow the plants to continue to grow to test for Roundup® sensitivity/resistance (optional).



Germination requires a humid environment.

The first true leaves may be visible 2 weeks after planting, depending on light and temperature conditions.

II. ISOLATE DNA FROM SOYBEAN AND FOOD PRODUCTS

Reagents	Supplies and Equipment
Soy or corn food products	Pellet pestles
Wild-type or Roundup Ready® soybean tissue	Permanent marker
Edward's buffer, 2.5 ml	1.5 ml microcentrifuge tubes
Isopropanol, 1 ml	Micropipet and tips (100-1000 µl)
Tris/EDTA (TE) buffer with RNase A, 300 µl	Microcentrifuge tube racks
	Microcentrifuge
	Vortexer (optional)
	Water bath or heating block (95-100°C)
	Container with cracked or crushed ice

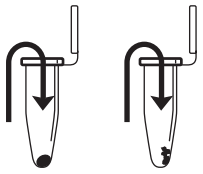
Your instructor will assign you either wild-type or Roundup Ready® soybean control.

1. Prepare tissue from wild-type *or* Roundup Ready® soybeans.

   a. From soy leaves: Cut two pieces of tissue approximately 1/4 inch in diameter. Place the leaf tissue in a clean 1.5 ml tube, and label with soybean type and your group number.

   b. From seed embryo: The embryo is a small (3 mm) flap located beside the eye spot (hilum) and underneath the seed coat. Gently remove the seed coat by rubbing the seed between your fingers. Remove the embryo flap and place it in a 1.5 ml tube. Label the tube with the soybean type and your group number.

The large end of a 1,000 µl pipet tip will punch disks of this size.



2. Prepare soy or corn food product. Crush a small amount of dry product on a clean piece of paper or in a clean plastic bag to produce a coarse powder. Add the crushed food product to a clean 1.5 ml tube to a level about halfway to the 0.1 ml mark. Label the tube, with the food type and your group number.

3. Add 100 µl of Edward's buffer to each tube containing the plant or food material.

4. Twist a clean pestle against the inner surface of the 1.5 ml tube to *forcefully* grind the plant tissue or food product for 1 minute.

The soy tissue sample should color the buffer green. Not all of the dry food will liquify.

5. Add 900 µl of Edward's buffer to each tube containing the ground sample. Grind briefly to remove tissue from the pestle.

6. Vortex the tubes for 5 seconds, by hand or machine.

7. Boil the samples for 5 minutes in a water bath or heating block.

This step denatures proteins, including DNA-digesting enzymes.

8. Place the tubes in a balanced configuration in a microcentrifuge, and spin for 2 minutes to pellet cell and food debris.

This step pellets insoluble material at the bottom of the tube.

9. Transfer 350 µl of each supernatant to a fresh tube. Maintain labels for each plant, food type, and group number. Be careful not to disturb

the pelleted debris when transferring the supernatant. Discard old tubes containing the precipitates.

This step precipitates nucleic acids, including DNA.

10. Add 400 µl of isopropanol to each tube of supernatant.

11. Mix by inverting the tubes several times, and leave at room temperature for 3 minutes.

The nucleic acid pellet may appear as a tiny teardrop-shaped smear or particles on the tube side. Don't be concerned if you can't see a pellet. A large or greenish pellet is cellular debris carried over from the first centrifugation.

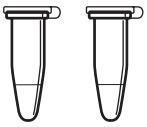
12. Place the tubes in a balanced configuration in a microcentrifuge, and spin for 5 minutes. Align tubes in the rotor with the cap hinges pointing outward. Nucleic acids will collect on the tube side under the hinge during centrifugation.

13. Carefully pour off the supernatant from each tube, then *completely* remove the remaining liquid with a medium pipet set at 100 µl.

Dry the pellets quickly with a hair dryer! To prevent blowing the pellet away, direct the air across the tube mouth, not into the tube.

14. Air dry the pellets for 10 minutes to evaporate remaining isopropanol.

15. Add 100 µl of TE/RNase A buffer to each tube. Dissolve the nucleic acid pellet by pipetting in and out. Take care to wash down the side of the tube underneath the hinge, where the pellet formed during centrifugation.



16. Incubate TE/RNAse A solution at room temperature for 5 minutes.

17. Microcentrifuge the tubes for 1 minute to pellet any material that did not go into solution.

You will use 2.5 µl of the DNA extract for the PCR reactions in Part III. The crude DNA extract contains nucleases that will eventually fragment the DNA at room temperature. Keeping the sample cold limits this activity.

18. DNA may be used immediately or stored at –20°C until you are ready to continue with Part III. Keep the DNA on ice during use.

III. AMPLIFY DNA BY PCR

Reagents	Supplies and Equipment
Food product DNA (from Part II) Wild-type *or* Roundup Ready® soybean DNA (from Part II) *35S primer/loading dye mix, 50 µl *Tubulin primer/loading dye mix, 50 µl Ready-To-Go™ PCR Beads Mineral oil, 5 ml (depending on thermal cycler) *Store on ice	Permanent marker Micropipet and tips (1-100 µl) Microcentrifuge tube rack 1.5 ml microcentrifuge tube Container with cracked or crushed ice Thermal cycler

Carry on with either wild-type or Roundup Ready® soybean control, as assigned by your instructor.

1. Set up 35S promoter reactions:

   a. Obtain 2 PCR tubes containing Ready-To-Go™ PCR Beads. Label with your group number.
   b. Label one tube "35S FP" (food product). Label another tube either

"35S WT" (wild-type soy plant) *or* "35S RR" (Roundup Ready® soy plant).

c. Use a micropipet with a fresh tip to add 22.5 µl of the 35S primer/loading dye mix to each tube. Allow several minutes for bead to dissolve.

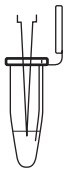
The primer loading dye mix will turn purple as the Ready-To-Go™ PCR Bead dissolves.

The tubulin gene is found in all plants and, so, is a positive control for the presence of amplifiable DNA.

d. Use a micropipet with a fresh tip to add 2.5 µl of food product DNA (from Part II) to the reaction tube marked "35S FP."

e. Use a micropipet with a fresh tip to add 2.5 µl of wild-type *or* Roundup Ready® soybean DNA (from Part II) to the appropriate reaction tube marked "35S WT" or "35S RR."

2. Set up tubulin reactions:



If the reagents become splattered on the wall of the tube, pool them by pulsing in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.

The mineral oil prevents the PCR mix from evaporating and condensing on the tube cap during cycling. Most modern thermal cyclers have heated lids that prevent condensing and DO NOT require the addition of mineral oil.

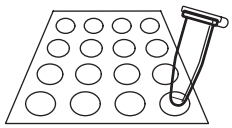
a. Obtain 2 PCR tubes containing Ready-To-Go™ PCR Beads. Label with your group number.

b. Label one tube "T FP" (food product). Label another tube either "T WT" (wild-type) or "T RR" (Roundup Ready®).

c. Use a micropipet with a fresh tip to add 22.5 µl of the tubulin primer/loading dye mix to each tube. Allow several minutes for bead to dissolve.

d. Use a micropipet with a fresh tip to add 2.5 µl of food product DNA (from Part II)) to the reaction tube marked "T FP."

e. Use a micropipet with a fresh tip to add 2.5 µl of wild-type or Roundup Ready® soybean DNA (from Part II) to the appropriate reaction tube marked "T WT" or "T RR."



3. If necessary, add one drop of mineral oil to the top of the reactants in the PCR tubes. Be careful not to touch the dropper tip to the tube or reactants, or subsequent reactions will be contaminated with DNA from your preparation.

4. Store samples on ice until you are ready to begin thermal cycling.

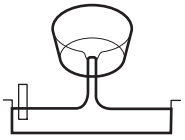
5. Program the thermal cycler for 32 cycles of the following profile. The program may be linked to a 4°C hold program after the 32 cycles are completed.

Denaturing step:	94°C	30 seconds
Annealing step:	60°C	30 seconds
Extending step:	72°C	30 seconds

6. After cycling, store the amplified DNA at –20°C until you are ready to continue with Part IV.

IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

Reagents	Supplies and Equipment
Food product PCR product (from Part III)	Micropipet and tips (1-100 µl)
Wild-type *or* Roundup Ready® soybean PCR product (from Part III)	1.5 ml microcentrifuge tube rack
*pBR322/*Bst*N1marker, 130 µl	Gel electrophoresis chamber
1X TBE, 300 ml	Power supply
2% agarose in 1X TBE, 50 ml	Staining trays
Ethidium bromide (1 µg/ml), 250 ml or	Latex gloves
*Carolina*BLU™ gel & buffer stain, 7 ml	UV transilluminator (for use with ethidium bromide)
*Carolina*BLU™ final stain, 250 ml	White light transilluminator (for use with *Carolina*BLU™)
*Store on ice	Camera
	Water bath (60°C)



Avoid pouring an overly thick gel, which is more difficult to visualize. The gel will become cloudy as it solidifies.

1. Seal the ends of the gel-casting tray with masking tape, and insert a well-forming comb.

2. Pour 2% agarose solution to a depth that covers about 1/3 the height of the open teeth of the comb.

3. Allow the gel to solidify completely. This takes approximately 20 minutes.

Do not add more buffer than necessary. Too much buffer above the gel channels electrical current over the gel, increasing running time.

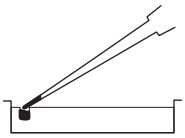
4. Place the gel into the electrophoresis chamber, and add enough 1X TBE buffer to cover the surface of the gel.

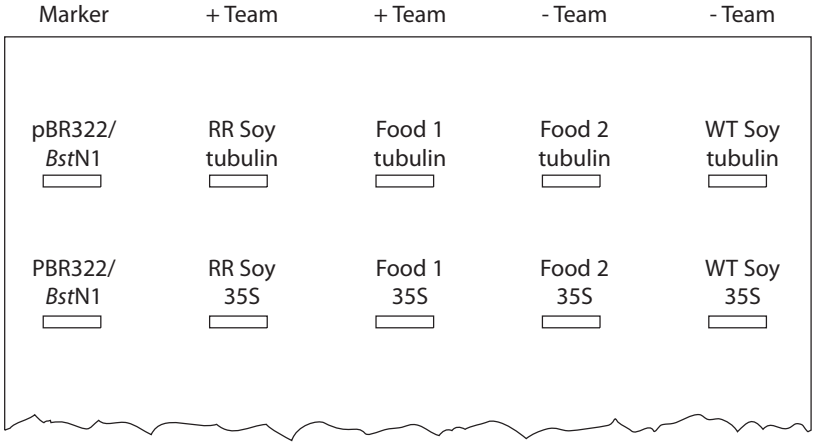
5. Carefully remove the comb, and add additional 1X TBE buffer to just cover and fill in wells, creating a smooth buffer surface.

Expel any air from the tip before loading. Be careful not to push the tip of the pipet through the bottom of the sample well.

6. Use a micropipet with a fresh tip to add 20 µl of each of the sample/loading dye mixtures into different wells of a 2% agarose gel, according to the following scheme. (If you used mineral oil during PCR, pierce your pipet tip through the layer of mineral oil to withdraw the PCR products, and leave the mineral oil behind in the original tube.)

100-bp ladder may also be used as a marker.





7. Load 20 µl of the molecular weight marker (pBR322/*Bst*N1) into one well.

8. Run the gels at 130 V for approximately 30 minutes. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

Destaining the gel for 5-10 minutes in tap water leeches unbound ethidium bromide from the gel, decreasing background and increasing contrast of the stained DNA.

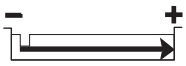
9. Stain the gel in ethidium bromide or *Carolina*BLU™:

   a. For ethidium bromide, stain 10-15 minutes. Decant stain back into sotrage container for resuse, and rinse gel in tap water. *Use gloves when handling ethidium bromide solution and stained or anything that has ethidium bromide on it. Ethidium bromide is a known mutagen and care should be taken when using and disposing of it.*

Transillumination, where the light source is below the gel, increases brightness and contrast.

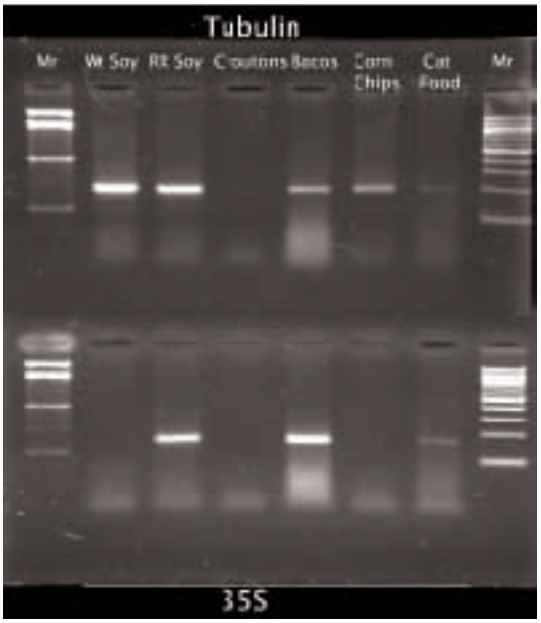
   b. For *Carolina*BLU™, follow directions in the Instructor Planning section.

10. View gel using transillumination, and photograph.



RESULTS & DISCUSSION

1. Observe the photograph of the stained gel containing your sample and those of other students. Orient the photograph with wells at the top. Interpret each lane of the gel. Use the sample gel pictured below to help you.



   a. Scan across the photograph of your gel and others as well to get an impression of what you see in each lane. You should notice that virtually all experiment lanes contain one or two prominent bands.

   b. Now locate the lane containing the pBR322/*Bst*N I marker on the left hand side of the gel. Working from the well, locate the bands corresponding to each restriction fragment: 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp (may be faint or not visible at all). Alternatively, locate the lane containing the 100-bp ladder on the right hand side of the gel. These DNA markers increase in size in 100 bp increments starting with the fastest migrating band of 100-bp.

   c. The amplification products of the 35S promoter (162 bp) and of the tubulin gene (187 bp) should align between the 121-bp and 383-bp fragments of the pBR322/*Bst*NI marker (or between the 100-bp and 200-bp markers of the 100-bp ladder).

   d. It is common to see a second band lower on the gel. This diffuse (fuzzy) band is "primer dimer," an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. Primer dimer is approximately 50 bp, and should be in a position ahead of the 121-bp fragment of the pBR322/*Bst*NI marker (or the 100-bp marker of the 100-bp ladder).

e. Additional faint bands, at other positions on the gel, occur when the primers bind to chromosomal loci other than 35S or tubulin, giving rise to "nonspecific" amplification products.

2. How would you interpret each of the following banding patterns:

162 bp (35S)	187 bp (tubulin)	~50 bp (primer dimer)
present	present	present
absent	present	present
absent	absent	present
absent	absent	absent

BIOINFORMATICS

Biological information is encoded in the nucleotide sequence of DNA. Bioinformatics is the field that identifies biological information in DNA using computer-based tools. Some bioinformatics algorithms aid the identification of genes, promoters, and other functional elements of DNA. Other algorithms help determine the evolutionary relationships between DNA sequences.

Because of the large number of tools and DNA sequences available on the Internet, experiments done *in silico* ("in silicon," or on the computer) now complement experiments done *in vitro* (in glass, or test tube). This movement between biochemistry and computation is a key feature of modern biological research.

In Part I you will use the Basic Local Alignment Search Tool (BLAST) to identify sequences in biological databases and to make predictions about the outcome of your experiments. In Part II you will discover some of the genes and functions that are transferred into GM plants.

I. Use *BLAST* to Find DNA Sequences in Databases (Electronic PCR)

The following primer sets were used in the experiment:

5'-CCGACAGTGGTCCCAAAGATGGAC-3'	(Forward Primer)
5'-ATATAGAGGAAGGGTCTTGCGAAGG-3'	(Reverse Primer)
5'-GGGATCCACTTCATGCTTTCGTCC-3'	(Forward Primer)
5'-GGGAACCACATCACCACGGTACAT-3'	(Reverse Primer)

1. Initiate a BLAST search.
   a. Open the Internet site of the National Center for Biotechnology Information (NCBI) www.ncbi.nlm.nih.gov/.
   b. Click on *BLAST* in the top speed bar.
   c. Click on *Nucleotide-nucleotide BLAST (blastn)*.
   d. Enter one of the primer sets into the *Search* window.
   e. Delete any non-nucleotide characters from the window.
   f. Click on *BLAST*!.
   g. Click on *Format!* and wait for your results.
2. The results of the *BLAST* search are displayed in three ways as you scroll down the page:
   a. A graphical overview illustrating how significant hits align with the query sequence,

b. a list of *significant alignments* with *Geneinfo Identifier* (*gi*) – links, and

c. a detailed view of the primer sequences (*query*) aligned to the nucleotide sequence of the search hit (*subject*).

3. What is the predicted length of the product that the primer set would amplify in a PCR reaction (*in vitro*)?

   a. In the list of significant alignments, notice the scores in the *E-Value* column on the right. The *Expectation* or *E-Value* is the number of alignments with the query sequence that would be expected to occur by chance in the database. The lower the *E-Value* the higher the probability that the hit is related to the query. For example, *3e-4* denotes $3x10^{-4}$ or 0.0003. Shorter queries, such as primers, produce higher *E-values*.

   b. Note any significant alignment that has an *E-Value* less than 0.1.

   c. Scroll down to the *Alignments* section, and examine the corresponding alignments with the two primers.

   d. The lowest and highest nucleotide positions in the subject sequence indicate the borders of the amplified sequence. *Calculate the length of the amplified sequence.*

   e. Don't forget to add one nucleotide to your result!

4. What DNA sequence does this primer set amplify? Is this the primer set to detect a GM product or the control primer set?

   a. Examine several of the search hits with the lowest *E Values*.

   b. Look at the datasheets reached by the *gi* – link on the left.

5. Repeat the electronic PCR to determine the DNA sequence amplified by the second gene with the second primer set and determine what gene this set amplifies.

II. Use *BLAST* to Identify Transgenes Driven by the 35S Promoter

1. Extract (copy) the sequence of the 35S amplicon (amplified product) from the appropriate *gi* datasheet in Part I. This is the entire sequence between the two primers.

2. Perform a *BLAST* search with this sequence.

3. Follow the *gi* – links for some of the hits and determine various genes that are linked to the 35S promoter – to be expressed in transgenic plants.

Instructor Information

REAGENTS, SUPPLIES & EQUIPMENT CHECK LIST

This experiment is available as a *DNA Learning Center Kit*, available ready-to-use from Carolina Biological Supply Company, catalog numbers 21-1366 through 21-1371. All kits include materials needed for DNA extraction and PCR amplification (for 0.2 ml or 0.5 ml PCR tubes); some kits contain additional materials for gel electophoresis and staining with ethidium bromide or *Carolina*BLU™. Visit the Carolina Biological Internet site at http://www.carolina.com/ or call 800-334-5551.

Reagents	Supplies and Equipment
Wild-type soybean seeds	Planting pot or flat
Roundup Ready® soybean seeds	Potting soil
Edward's buffer	Micropipets and tips (1-1,000 µl)
Soy or corn food products	1.5 ml microcentrifuge tubes
100% Isopropanol	Microcentrifuge tube racks
Tris/EDTA (TE) buffer with RNase A	Microcentrifuge for 1.5-ml tubes
35S primer/loading dye mix*	Pellet pestles
Tubulin primer/loading dye mix*	Water bath or heating block (95-100°C)
Ready-to-Go™ PCR Beads	Thermal cycler
Mineral oil (depending on thermal cycler)	Electrophoresis chambers
DNA marker pBR322/*Bst*NI (0.075 µg/µl)*	Electrophoresis power supplies
Agarose	Latex gloves
1x TBE electrophoresis buffer	Staining trays
Ethidium bromide solution, 1 µg/ml *OR*	UV transilluminator (ethidium bromide staining)
*Carolina*BLU™ gel/buffer stain	White light box (*Carolina*BLU™ staining)
*Carolina*BLU™ final stain	Camera or photo-documentary system
Roundup herbicide (optional)	Permanent markers
	Container with cracked or crushed ice
	Water bath for agarose (60°C)
	Vortexer (optional)

*Store at –20°C

Ready-to-Go™ PCR Beads incorporate *Taq* polymerase, dNTPs, and $MgCl_2$.
Each bead is supplied in an individual 0.5-ml tube or a 0.2-ml tube.

CONCEPTS AND METHODS

This laboratory can help students understand several important concepts of modern biology:
- The relationship between genotype and phenotype.
- Forensic identification of genes.
- Methods for producing transgenic crops.
- The movement between *in vitro* experimentation and *in silico* computation.

The laboratory uses several methods for modern biological research:
- DNA extraction and purification.
- Polymerase chain reaction (PCR).
- Gel electrophoresis.
- Bioinformatics.

INSTRUCTOR PLANNING

The following table will help you to plan and integrate the four parts of the experiment.

Part		Day	Time	Activity
I.	Plant soybean seeds	2-3 weeks before lab	15-30 min.	Plant soybean seeds
II.	Isolate DNA	1	30 min.	**Pre-lab:** Set up student stations
			30-60 min.	Isolate soy DNA
III.	Amplify DNA by PCR	2	30-60 min.	**Pre-lab:** Set up student stations
			15-30 min.	Set up PCR reactions
			70+ min.	**Post-lab:** Amplify DNA in thermal cycler
IV.	Analyze Amplified DNA by Gel Electrophoresis	3	30 min.	Prepare agarose gel solution and cast gels
		4	30 min	Load DNA samples into gels
			30+ min.	Electrophorese samples
			20+ min.	**Post-lab:** Stain gels
			20 min. to overnight	**Post-lab:** De-stain gels
			20 min.	**Post-lab:** Photograph gels

I. Plant Soybean Seed

Tissue from wild-type and Roundup Ready® soybean plants are used as negative and positive controls of the experiment. *Soybean seeds must be planted 2-3 weeks before the date anticipated for DNA extraction and amplification by PCR.* Plant tissue may be harvested for DNA isolation at any point after plantlets emerge from the soil. Two 1/4 inch diameter leaf disks are required for each experiment. Several wild-type and Roundup Ready® plants can be set aside for treatment with Roundup to test for herbicide sensitivity/resistance.

The following Carolina products are suggested for growing soybean seedlings:

Standard Poly-Tray without Holes (54 x 27 x 6 cm tray)	*item number 66-5666*
Poly-Flats (6-cm deep cells that can be separated into individual pots):	
8-Cell Tray	*item number 66-5668*
24-Cell Tray	*item number 66-5669*
36-Cell Tray	*item number 66-5670*
Redi-Earth Soil (8 lb. Bag)	*item number 15-9701*

II. Isolate DNA from Soybean and Food Products

Assign each team a number at the outset of the experiment. This will make it easier to mark and identify the several types of small test tubes used in the experiment.

Have students bring in foods they want to test for transgenes. Fresh or dry food products work well with the DNA extraction protocol outlined below. Food products should contain either soy or corn as an ingredient. Products that have been tested successfully using this procedure include corn and tortilla chips, artificial bacon bits, corn muffin mix, granola and energy bars, protein powder, and pet food.

If extracting DNA from seed embryos, soak the wild-type and Roundup Ready® soybean seeds in separate containers of distilled water for a minimum of 30 minutes. This will soften the seeds, making the embryos easier to remove.

Each lab team will set up four of six kinds of reactions. Each team will test a food product of their choice, while half of the teams will set up either a positive ("+") or negative ("-") control, according to the scheme below:

Test Item	35S Primers	Tubulin Primers	Teams
Soy or corn food product	✓	✓	All
Wild-type soybean tissue	✓	✓	-
Roundup Ready® soybean tissue	✓	✓	+

The cells walls of living plant tissue and the granular structure of dried foods typically are broken up by grinding with a mortar and pestle. This can be accomplished directly in a 1.5 ml tube using a plastic pestle. A no-cost pestle can be made by heating a 1,000 µl pipet tip in a gas flame until it just melts. Then force the melted tip into a 1.5 ml tube and twist to obtain a smooth surface.

Set up a 95°C heating block, or one boiling water bath per 12 samples. A boiling water bath can be made in one of two ways:

- Place tubes in a floating test tube rack within a beaker of water atop a hot plate. Regulate temperature to maintain a low boil.
- Fill a beaker with water and cover tightly with a double layer of aluminum foil. Use a pencil to punch holes to hold the tubes, and maintain at low boil with a hot plate.

Watch out for lids opening as the tubes heat.

Pre-lab Set Up (per student team)

Soy or corn food product
Wild-type or Roundup Ready® soybean tissue (leaf or embryo)
Edward's buffer, 2.5 ml
Isopropanol, 1 ml
Tris/EDTA (TE) buffer with RNase A, 300 µl (thaw and store on ice)

1.5 ml microcentrifuge tubes
Permanent marker
Pellet pestles
Micropipet and tips (100-1,000 µl)
Microcentrifuge tube rack
Container with cracked or crushed ice

Shared Items

Microcentrifuge
Water bath or heating block (95-100°C)
Vortexer (optional)

III. AMPLIFY DNA BY PCR

Two PCR reactions are performed for each plant or food sample. One primer set amplifies the 35S promoter from cauliflower mosaic virus. The presence of a 35S product is diagnostic for the presence of a transgene, since the 35S promoter is used to drive expression of the glyphosate (Roundup®) resistance gene or *Bt* gene in edible crops. A second primer set amplifies a fragment of a tubulin gene and controls for the presence of plant template DNA. Since the tubulin gene is found in all plant genomes, the presence of a tubulin product indicates amplifiable DNA in the sample isolated. The following table interprets the possible combinations of results for 35S and tubulin amplifications from soybeans and food products.

Plant or plant product	35S Promoter	Tubulin	Interpretation
Wild-type soybean	Negative	P ositive	Transgene absent
Roundup Ready® soybean	Positive	Positive	Transgene present
Product A	Negative	P ositive	Transgene absent
Product B	P ositive	Positive	Transgene present
Product C	Negative	Negative	DNA template absent

Each Ready-To-Go™ PCR Bead contains reagents so that when brought to a final volume of 25 ml the reaction contains 2.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, and 200 mM of each dNTP.

The lyophilized *Taq* DNA polymerase in the Ready-To-Go™ PCR Bead becomes active immediately upon addition of the primer/loading dye mix. In the absence of thermal cycling, "nonspecific priming" at room temperature allows the polymerase to begin generating erroneous products, which can show up as extra bands in gel analysis. *Therefore, work quickly. Be sure the thermal cycler is set and have all experimenters set up their PCR reactions as a coordinated effort. Add primer/loading dye mix to all reaction tubes, then add each student template, and begin thermal cycling as quickly as possible. Hold reactions on ice until all are ready to load into the thermal cycler.*

Each primer/loading dye mix incorporates the appropriate primer pair (0.25 picomoles/µl of each primer), 13.9% sucrose, and 0.0082% cresol red. The inclusion of loading dye components, sucrose and cresol red, allows the amplified product to be directly loaded into an agarose gel for electrophoresis. The primer/loading dye mix may collect in the tube caps during shipping; pool the reagent by spinning the tubes

briefly in a microcentrifuge or by tapping the tube ends on the desktop.

PCR amplification from crude cell extracts is biochemically demanding, and requires the precision of automated thermal cycling. However, amplification of the 35S and tubulin loci is not complicated by the presence of repeated units. Therefore, the recommended amplification times and temperatures will work adequately for all types of thermal cyclers.

Pre-lab Set Up (per student team)

Food Product DNA, from Part II (store on ice)
Wild-type or Roundup Ready® soybean DNA, from Part II (store on ice)
50 µl 35S primer/loading dye mix (thaw and store on ice)
50 µl tubulin primer/loading dye mix (thaw and store on ice)
4 Ready-To-Go™ PCR Beads (in PCR tubes)
Permanent marker
Micropipet and tips (1-100 µl)
Microcentrifuge tube rack
Mineral oil, 5 ml (depending on thermal cycler)
Container with cracked or crushed ice

Shared Item
Thermal cycler

IV. ANALYZE AMPLIFIED DNA BY GEL ELECTROPHORESIS

Prepare a 1X concentration of TBE by adding 75 ml of 20X concentrated stock into 1,425 ml of deionized or distilled water. Mix thoroughly.

Prepare a 2% agarose solution by adding 2 g of agarose to 100 ml of 1X TBE in a 500 ml flask or beaker. Heat the flask or beaker in a boiling water bath (approximately 15 minutes) or in a microwave oven (approximately 4 minutes) until the agarose is *completely* dissolved. You should no longer see agarose particles floating in solution when the beaker is swirled. Allow the agarose to cool to approximately 60°C, and hold at this temperature in a hot water bath. Cover beaker or flask with aluminum foil, and skim any polymerized "skin" off the top of the solution before pouring.

The cresol red and sucrose in the primer mix function as loading dye, so that amplified samples can be loaded directly into an agarose gel. This is a nice time saver. However, since it has relatively little sugar and cresol red, this loading dye is more difficult to use than typical loading dyes. So, encourage students to load carefully.

Plasmid pBR322 digested with the restriction endonuclease *Bst*NI is an inexpensive marker and produces fragments that are useful as size markers in this experiment. The size of the DNA fragments in the marker are 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp. Use 20 µl of a 0.075 µg/µl stock solution of this DNA ladder per gel. Other markers or a 100 bp ladder may be substituted.

View and photograph gels as soon as possible after appropriate staining/destaining. Over time, at room temperature, the small-sized PCR products will diffuse through the gel and lose sharpness.

Pre-lab Set Up (per student lab team)

Wild-type soy 35S and tubulin PCR products from Part III (store on ice)
Roundup Ready® soy 35S and tubulin PCR products from Part III (store on ice)
Food product 35S and tubulin PCR products from Part III (store on ice)
pBR322/*Bst*NI markers (thaw and store on ice)
2% agarose in 1X TBE (hold at 60°C), 50 ml per gel
1X TBE buffer, 300 ml per gel
Ethidium bromide (1 µg/ml), 250 ml
or
*Carolina*BLU™ gel & buffer stain, 7 ml
*Carolina*BLU™ final stain, 250 ml

Micropipet and tips (1-100 µl)
Microcentrifuge tube rack
Electrophoresis chamber and power supply
Latex gloves
Staining tray
Container with cracked or crushed ice

Shared Items

Water bath for agarose solution (60°C)
Transilluminator with camera

*Carolina*BLU™ STAINING

Post-Staining

1. Cover the electrophoresed gel with the *Carolina*BLU™ Final stain and let sit for 20-30 minutes. Agitate gently (optional).

2. After staining, pour the stain back into the bottle for future use. (The stain can be used 6-8 times.)

3. Cover the gel with deionized or distilled water to destain. Chloride ions in tap water can partially remove the stain from the DNA bands and will cause the staining to fade.

4. Change the water 3-4 times over the course of 30-40 minutes. Agitate the gel occasionally.

5. Bands that are not immediately present will become more apparent with time and will reach their maximum visibility if the gel is left to stain overnight in just enough stain to cover the gel. Gels left overnight in a large volume of water may destain too much.

Pre-Staining

*Carolina*BLU™ can also be used to stain the DNA while it is being electrophoresed. Pre-staining will allow students to visualize their results prior to the end of the gel run. However, post-staining is still required for optimum viewing.

To pre-stain the gel during electrophoresis, add *Carolina*BLU™ Gel and Buffer Stain in the amounts indicated in the table below. Note that the amount of stain added is dependent upon the voltage used for electrophoresis. *Do not use more stain than recommended. This may precipitate the DNA in the wells and create artifact bands.*

Gels containing *Carolina*BLU™ may be prepared one day ahead of the lab day, if necessary. However, gels stored longer tend to fade and lose their ability to stain DNA bands during electrophoresis.

Use the table below to add the appropriate volume of *Carolina*BLU™ stain to the agarose gel:

Voltage	Agarose Volume	Stain volume
<50 Volts	30 ml	40 µl (1 drop)
	200 ml	240 µl (6 drops)
	400 ml	520 µl (13 drops)
>50 Volts	50 ml	80 µl (2 drop)
	300 ml	480 µl (12 drops)
	400 ml	640 µl (16 drops)

Use the table below to add the appropriate volume of *Carolina*BLU™ stain to 1X TBE buffer:

Voltage	Agarose Volume	Stain volume
<50 Volts	500 ml	480 µl (12 drop)
	3000 ml	3 ml (72 drops)
>50 Volts	500 ml	960 µl (24 drop)
	2600 ml	5 ml (125 drops)

ANSWERS TO DISCUSSION QUESTIONS

How would you interpret each of the following banding patterns:

162 bp (35S)	187 bp (tubulin)	~50 bp (primer dimer)	
present	present	present	Transgene present
absent	present	present	Transgene present
absent	absent	present	No template DNA
absent	absent	absent	No PCR reaction