Detection of Genetically Modified Food

Teacher Notes for the Wet lab

Synopsis

Students perform DNA isolation on food products (corn or soy / organic and nonorganic) and DNA amplification by polymerase chain reaction (PCR) on food DNA to detect the presence of genetic modification. The students will use maize reference standards for genetically modified DNA as controls and samples will be analyzed using agarose gel electrophoresis.

Appropriate Grade Level

High School: Advanced

Two-year college: Intermediate, Advanced

Four-year college: Intermediate, Advanced

This laboratory activity was designed and written primarily for the training of two-year college biotechnology technicians. The lab has been incorporated into the curriculum of the Molecular Biology I course at Madison Area Technical College, Madison, WI.

Objectives

At the end of this lab, students will be able to:

Discuss a method for detecting genetic modification in food.

Describe and perform a method of isolating DNA.

Describe and perform a method of amplifying DNA.

Describe and perform a method of separating DNA by size using agarose gel electrophoresis.

Operate basic laboratory equipment including: micropipets, balance, centrifuge, gel electrophoresis, vacuum manifold, thermalcycler, UV transilluminator, camera, and waterbath.

Use protective equipment.

Comprehend a technical vocabulary.

Follow a protocol.

Keep accurate records.

Summarize the results of the lab activity.

Relate the concepts of this lab activity to current food and health issues.

Construct or design extensions of this lab activity.

Getting Ready

Length of the Lab

A suggested time allotment follows:

Day 1 (45 min): Introduction and selection of foods to be tested.

Day 2 (45 min): Isolate DNA from food. (If steps 1-5 are prepared ahead of time)

Day 3 (45 min): Set up and run polymerase chain reaction.

Day 4 (45 min): Load and run agarose gels, stain and photograph

Day 5 (45 min): Interpret results

Student Team Logistics

Organize the class into teams of 3-4 students.

Have each team bring in four food samples for testing (ex. corn meal, corn tortilla chips, tofu, corn muffin mix). Have one team prepare one 2% positive control standard sample and one 0% control standard sample. There should be plenty of isolated standard DNA to share with all the other groups.

Preparation Time Required

10-15 hours depending if all reagents are on hand.

Equipment and Materials Needed to Prepare and Run This Lab

Equipment

Micropipettors 100 - 1000 µL, 10 - 100 µL, and 0.5 - 10 µL
Analytical balance
Autoclave
pH meter
Spatulas
Thermometer
55-60º C incubator
70ºC waterbath or dri bath
Timers
Vortexer
Microcentrifuge 14,000 x g
Minifuge
Microcentrifuge tube racks
Vac-Man® Laboratory Vacuum Manifold or syringes (see Preparation Tips #2)
Vacuum line or vacuum pump
Lab markers
Thermalcycler
Horizontal gel electrophoresis boxes
Gel electrophoresis power supplies

UV transilluminator
Staining trays
Camera
Assorted beakers, graduated cylinders and storage bottles
Stir bars
Magnetic stirplate
Hotplate or microwave oven
Heat-proof gloves
Refrigerator
Freezer
Goggles or safety glasses

Materials

Institute for Reference Materials and Measurements (IRMM)-certified reference standards obtained from

- Maize Powder MZ-Set - Sigma-Aldrich. #78761 *OR*
- Individual IRMM certified reference standards
  Sigma-Aldrich 0% cat. #77119 and 2% cat #79112

Dried food products containing corn meal or soy flour examples include: corn meal, corn flour, corn chips, soy flour, organic corn meal, or organic soy flour.

Microcentrifuge tubes 1.5 mL (sterile)
Sterile sticks Fisher 01-340
Nuclease free water Promega P1193
Weigh boats
Aerosol resistant pipet tips
100 - 1000 µL, 10- 100 µL, and 0.5 - 10 µL
Tris-HCl Sigma T 5941
NaCl Sigma S 9888
EDTA (disodium salt) Sigma E1644
SDS Sigma L 4509
Guanidine-HCl Sigma G 9284
Proteinase K Promega V3021
Labeling tape
3 ml syringes (sterile) if you do not have access to the vacuum manifold see Preparation Tips #2
Wizard® minicolumns Promega A7211
Wizard® resin Promega A7181
Isopropanol Sigma I 9516
PCR primers: CaMV35S Promoter
Ready-To-Go™ PCR beads Amersham Pharmacia 27-9555-01
Sterile mineral oil Sigma M 5904
4% precast agarose gels BioWhittaker Molecular Applications 54926
You may prepare you own 2% agarose gel see Preparation Tips #4
TBE electrophoresis buffer Sigma T 4415
PCR Marker, 50-2000 bp Novagen 69278-3
Blue/Orange Loading Dye, 6X Promega G1881

Deionized or distilled water
Film
Ethidium bromide 10 mg/ml Sigma E 1510
Gloves
Lab coats or aprons

Equipment and Materials for Each Team of Four Students

Equipment
Micropipettors 100 - 1000 µL, 10 - 100 µL, and 0.5 - 10 µL
Timer
Microcentrifuge tube rack
Lab marker
Horizontal gel electrophoresis box
Staining tray
Goggles or safety glasses

Materials
Certified references for the positive and 0% control
Food products (4) organic or nonorganic containing corn or soy products
Several Microcentrifuge tubes 1.5 mL (sterile)
4 Sterile sticks (1 for each food sample and controls)
4 mL nuclease free water
Weigh boats
Aerosol resistant pipet tips 100 - 1000 µL, 10 - 100 µL, and 0.5 - 10 µL
6 mL extraction buffer
700 µl 5M guanidine-HCl
300 µl 20 mg/mL proteinase K
Labeling tape
6 - 3mL syringes
6 Wizard® minicolumns
6 mL Wizard® resin
12 mL 80% isopropanol
7 µl of each PCR primer
7 Amersham Pharmacia Ready-To-Go™ PCR beads
1X TBE electrophoresis buffer
Agarose to make gel
6 µl PCR Markers
20 µl Blue/Orange Loading Dye, 6X
100 mL 1µg/mL ethidium bromide stain
Gloves, several pair per student
Lab coat or apron, 1 per student

Safety Procedures

- Wear safety goggles, closed toe shoes, lab coats, and disposable gloves at all times during the lab.

- Wash hands before and at the conclusion of the lab
- If the power is on, electrical shock may result from touching the buffer or electrophoresis equipment.
- Never leave the electrophoresis power unit on without supervision. There is a risk of fire if the buffer leaks out or if the buffer should evaporate completely during electrophoresis.
- Never leave stir plates or hotplates on without supervision.
- Be sure that students are familiar with the operating instructions and safety precautions before they use any centrifuge. These machines can be very hazardous if handled improperly.
- Use caution with hot liquids and glassware. Wear heat-proof gloves when melting agarose.
- Exercise caution when working near hot water baths and thermalcycler.
- Ethidium bromide is a mutagen and cancer-suspect agent. Wear gloves when handling the stain and stained gels. Designate an ethidium bromide stain work area. Dispose of stain properly see *DNA Science* by Micklos and Freyer, pp.256-257. In high school classrooms the teacher should handle the ethidium bromide stain.
- Review all MSDS (Material Safety Data Sheets) for all chemicals and reagents in the lab before preparing and running the lab.
- Never look at an unshielded UV light source. Wear a full face UV shield and cover all exposed skin if your UV light source is unshielded.

Directions for Setting Up the Lab

Solution Preparation

Extraction buffer

10 mM Tris-HCl at pH 7.5
150 mM NaCl
2mM EDTA
1% SDS
Store at room temperature for a maximum of three months.

5M Guanidine-HCl

Add 80 mL of distilled water to 47.8 g of guanidine hydrochloride in a flask, stir until completely dissolved. BTV to 100 mL with distilled water and autoclave. Store at room temperature for a maximum of three months.

20mg/mL Proteinase K

Add 5 mL nuclease-free water to 100 mg Proteinase K in a sterile tube or flask. Aliquot and store at -20ºC for a maximum of six months.

80% Isopropanol

80 mL of 100% isopropanol
20 mL sterile distilled water
Makes 100 mL

Oligonucleotide Primer Sequences

Primers are available from several companies and prices vary.

Dilute primers to a final concentration of 25 pmol/µL in nuclease free water. Primer stocks may be stored frozen for one year.

CaMV 35S Promotor
Sense: 5' **GCT CCT ACA AAT GCC ATC A** 3'
Antisense: 5' **GAT AGT GGG ATT GTG CGT CA** 3'

TBE electrophoresis buffer

Follow manufacturer directions for 1X solution.

PCR Marker

Use 6 µl per gel lane. Store in refrigerator.

Ethidium Bromide stain 1 µg/mL

Add 50 µL of 10mg/mL ethidium bromide to 500 mL of deionized or distilled water. Store in unbreakable opaque bottles at room temperature. Label bottle <u>Caution: Ethidium Bromide</u>

Preparation Tips

For help determining how to reconstitute your oligoneucleotide primer sequences. See the following web sites: www.lifetech.com TechOnLine, FAQs – Calculations for Custom Oligos or www.genosys.com Custom Oligos, FAQ – You And Your Oligos

If you don't use the Amersham Pharmacia Ready-To-Go™ PCR beads prepare the following PCR master mix. Note that the primer concentrations are different.

	Single PCR (µL)	10 Reactions (µL)
DNA template	5	--
Primer 1 (50 pmol/µL)	1	10
Primer 2 (50 pmol/µL)	1	10
10X Reaction Buffer	10	100
$MgCl_2$ (25mM Solution)	6	60
PCR Nucleotide Mix (10 mM)	2	20
Taq DNA Polymerase (5U/µL)	0.5	5
NucleaseFree Water	74.5	745
Total Volume	100	950

- Thermalcycler Profile for PCR Using CaMV35S
  Forty cycles takes approximately 2.5 hr to complete with the Perkin Elmer Thermalcycler 480.

	35S Primer
Denaturation	3 min/94˚C
Amplification	20 sec/94˚C
	40 sec/54˚C
	60 sec/72˚C
# of cycles	40
Final extension	3 min/72˚C

Preparation Tips for successful PCR

1. Crush food products like corn chips before the lab so they are easier to weigh.
2. If you don't have access to a vacuum source. There is an alternative to the Vac-Man® Laboratory Vacuum Manifold. Use 3 mL syringes and plungers instead.
3. If you don't have access to a thermalcycler. Three waterbaths may be used as a substitute, with the students moving the samples between the different waterbaths set at three different temperatures.
4. If you can't purchase 4% precast agarose gels make 2% agarose gels in 1X TBE buffer instead.
5. If you can't use ethidium bromide stain, alternatives include *Carolina*BLU™ DNA Stain, Ward's DNA Stain and Edvotek® DNA InstaStain™. When using a stain with

less sensitivity than ethidium bromide, load all of the PCR DNA sample into the gel wells.

6. For information on the disposal of ethidium bromide stain consult (Micklos 1990) or (Horn 1993).
7. On day two the instructor may need to prepare the food samples ahead of time for steps 1-5 of the DNA Isolation portion, if class time is limited.
8. Use aerosol resistant pipet tips for all pipetting to prevent cross contamination between samples.
9. Highly processed foods such as oils or some cereals contain less and poorer quality DNA than less processed foods.
10. The Institute for Reference Materials and Measurements (IRMM)-certified reference standards Maize Powder MZ-Set contains at least 1 g of each four standards: 0%, 0.1%, 0.5% and 2% GMO dry powder. Individual IRMM certified reference standards may be purchased from Sigma-Aldrich, 0% cat. #77119 and 2% cat #79112.
11. Blue/Orange Loading Dye, 6X is a convenient marker dye containing orange G, bormophenol blue and xylene cyanol. The dye is provided in a pre-mixed ready-to-use form. Store at –20 ˚ C.
12. A few minutes after the power supply is turned on and current is applied to the gel, the loading dye should be seen moving toward the positive electrode end of the gel box. The Blue/Orange loading dye will eventually separate into three band colors. The yellowish orange band is orange G. The purplish band is bromophenol blue and the aqua colored band is xylene cyanol. Bromophenol blue dye migrates at approximately the same rate as a DNA fragment of 300 bp and xylene cyanol migrates at approximately the same rate as a DNA fragment of 9000 bp.
13. When aliquoting small amounts of liquid for students, each aliquot should contain slightly more than will be required for the lab. To get the contents of the tube in the bottom of the tube, tap the tube on the counter top or centrifuge for a couple seconds.
14. The instructor may want to consider dispensing the primers into each students sample tube, due to the very small quantity and cost of aliquoting extra amounts.
15. Prepare negative control samples for the PCR portion of the lab. A negative control is a check on the reagents used in this lab activity. Prepare the sample exactly the same as the others but do not add DNA. Make up the volume difference with water. If a PCR product is present after the amplification, foreign DNA has contaminated the reagents.
16. The PCR Marker consists of eight fragments that range in size from 50 – 2000 bp.
17. PCR trouble shooting hint: make sure your thermalcycler is calibrated.
18. Ask the students to bring in a variety of dried foods containing corn. Some examples are corn meal, corn muffin mix, corn chips, taco shells, pancake mix, organic chips, organic corn meal and anything else that might contain corn. Have students make a chart of all the samples being tested and controls to record the results of the class.

Reagent and Equipment Vendors

www.sigma-aldrich.com
www.promega.com
www.vwrsp.com
www.lifetech.com
www.novagen.com
www.edvotek.com
www.fotodyne.com
www.apbiotech.com
www.fishersci.com
www.nabt.org
www.genosys.com
www.carolina.com
www.wardsci.com

Student Lab Protocol-Wet Lab

graphic taken from
Science Education Partnership
Fred Hutchinson Cancer Research
Center

DNA Isolation

1. Wearing gloves, select your food sample or control sample. Weigh out 0.1g of food and place it into a disposable microcentrifuge tube.
2. Add 200µL of nuclease free water to the tube.
3. Use a sterile stick to homogenize the food sample to a smoot
4. Add 860µL of extraction buffer, 100µL of 5M Guanidine-HCl and 40µL of 20mg/mL Proteinase K to the tube containing the homogenate. Vortex tube.
5. Incubate at 55-60ºC for 3 hours with intermittent mixing.
6. Allow samples to cool at room temperature for 10 minutes.
7. Centrifuge 10 minutes at 14,000 x g in a microcentrifuge.
8. For each sample, attach one labeled 3mL syringe barrel to the Luer-Lok® extension of a Wizard® minicolumn and attach this minicolumn/syringe barrel assembly to the Vac-Man® Laboratory Vacuum Manifold.
9. Check to ensure all stopcocks are closed before proceeding.
10. Add 1mL of Wizard® resin to each minicolumn/syringe assembly.
11. Carefully remove 300µl of the cleared supernatant from each sample and transfer it to the barrel of the minicolumn/syringe assembly containing the Wizard® resin.
12. Open the stopcocks and apply a vacuum to pull the resin/supernatant mix into the minicolumn. When the entire sample has passed through the column, close the stopcock and turn off the vacuum. In this step the DNA will stick to the column.
13. Add 2mL of 80% isopropanol to each minicolumn and reapply the vacuum to draw the solution through the minicolumn. This step washes the column.
14. Remove the syringe barrel and transfer the minicolumn to a 1.5mL microcentrifuge tube. Centrifuge the minicolumn at 10,000 x g in a microcentrifuge for 2 minutes to remove any residual alcohol.
15. Transfer the minicolumn to a new microcentrifuge tube, add 50µl of 70ºC nuclease free water to the column and allow it to interact with the resin for 1 minute. This step elutes the DNA from the column.
16. Elute the DNA by centrifugation at 10,000 x g for 1 minute in a microcentrifuge.
17. You may stop here and store the DNA in the refrigerator for about a week. For longer periods, store it in the freezer.

PCR DNA Amplification

1. Wearing gloves obtain one Ready-To-Go™ PCR bead in a 0.5mL tube for each sample to be tested. Check that the bead in each tube is visible at the bottom of

the tube. Label the tube and place it in a rack. See Setting Up Reactions (Table 3).

2. Add 5µL of template DNA to the tube. Change pipet tips between samples.
3. Add 2µL of primer mix to the tube.
4. Add 18µL of nuclease free water. Cap the tube and gently vortex, then centrifuge briefly to collect the contents at the bottom of the tube. The total volume in the tube should be 25µL.
5. Overlay the reaction with 1 drop of mineral oil, if it is required for your thermalcycler.
6. Place your tubes in the thermalcycler and start the reaction. While you are waiting for the amplification reaction you may begin to prepare your agarose gel for analysis. When the reaction is complete. You may stop and freeze the reaction tubes or continue with gel electrophoresis.

Setting Up Reactions

For each group:

Tube #	Tube w/bead	DNA	Primer mix	H_2O	Mineral Oil	Gel Lane #
Sample #1	✓	5 µL	2 µL	18 µL	1 drop	
Sample #2	✓	5 µL	2 µL	18 µL	1 drop	
Sample #3	✓	5 µL	2 µL	18 µL	1 drop	
Sample #4	✓	5 µL	2 µL	18 µL	1 drop	
0% Standard	✓	5 µL	2 µL	18 µL	1 drop	
2% Standard	✓	5 µL	2 µL	18 µL	1 drop	
Neg. Control = NO DNA	✓	0 µL	2 µL	23 µL	1 drop	

Table 3.

Agarose Gel Electrophoresis

1. Wearing gloves obtain one 4% agarose TBE gel with eight wells, a gel box containing TBE 1X buffer, and a power supply.
2. Add 5µL loading dye to the tubes with your PCR samples, standards and negative control.

3. Load 15 µL of each sample, standard and negative control into separate wells of your gel, avoid mixing mineral oil with the sample. Save a lane for the PCR marker on each gel. Record where each sample is located on the gel.
4. Add 1µL loading dye to your PCR marker. Load the marker into one well of the gel.
5. Attach the gel box to the power supply, turn the power on, and set to 100-150 volts. Electrophorese for 40-60 minutes or until the bromophenol blue band has traveled one-third the length of the gel. Volt settings and time will vary with different equipment.
6. Wearing gloves, carefully remove the gel and put it into a staining tray. Cover the gel with ethidium bromide stain and stain for 5 -10 minutes.
7. After staining, decant the ethidium bromide stain from the staining tray back into the storage bottle.
8. Rinse the gel with tap water, in the tray, for several minutes to remove background ethidium bromide stain from the gel.
9. View on ultraviolet transilluminator and photograph. Record your results and share with all other groups.

Figure 2.

Sample Results

Figure 2. Lane 1) Negative Control. Lane 2) 0% Standard. Lane 3) 2% Standard, band at 195bp in length indicates genetic modification by presence of the CaMV35S promoter. Lane 4) PCR Marker; bottom to top: 50bp, 150 bp, 300 bp, 500 bp, 750 bp, 1000 bp, 1500 bp, and 2000 bp. Lane 5) Brand Name Corn Chip. Lane 6) Organic Corn Meal. Lane 7) Brand Name Corn Meal. Lane 8) Brand Name Organic Tortilla Chip.

Student Worksheet

Data Analysis and Interpretation Assessment

What samples or controls are in each lane of your gel?

Lane 1

Lane 2

Lane 3

Lane 4

Lane 5

Lane 6

Lane 7

Lane 8

Attach Gel Photo Here

1. What percentage agarose is the gel?
2. How long did the electrophoresis take?
3. What voltage or milliamp setting did you use?
4. How long did you stain the gel?
5. Can you see the PCR Marker on the gel?
6. Are all PCR Marker bands visible?
7. How many PCR Marker bands should there be and what size?
8. How many lanes show PCR bands?
9. Does the 0% standard show a PCR band?
10. Does your negative control show a PCR product band?
11. Does you positive control 2% standard show a PCR product band?
12. Are any bands in the 180-195 bp range?
13. Which lanes?
14. Do any organic samples show any PCR bands?
15. Under new federal organic labeling laws organic food may contain up to 5% nonorganic components. If cornmeal labeled organic contained 3% genetically modified corn would you be able to detect it with this detection method?

16. Design for your next experiment. If you had unlimited resources, how would you design a new experiment based on data you gathered or questions you asked during the course of your lab activity? Write your procedure in a numbered list.