

2025 BIORETS Curricular Materials

Title of the Lesson Plan: Genetically Modified Organisms (GMO) Investigation
BIORETS Teacher's Name: Patricia Hebenstreit
Intended School Year and Marking Period: 2026 first marking period
Subject and Grade Level: Biology, High School (Grades 9-12) – Farm-to-Table
Overview: <p>This lesson plan engages high school students in a hands-on investigation of genetically modified organisms (GMOs) using the Bio-Rad GMO Investigator™ kit. Students will explore DNA extraction, polymerase chain reaction (PCR), and agarose gel electrophoresis to determine whether common grocery store foods contain GMO-derived DNA. The lesson addresses biotechnology principles, the benefits and concerns of GMOs, and their impact on agriculture and society. Through laboratory activities, discussions, and analysis, students will develop critical thinking skills and understand the scientific processes behind GMO detection.</p>
Essential Standards: <ul style="list-style-type: none">• HS-LS1-1: From Molecules to Organisms: Structures and Processes• 2-LS4-1: Biological Evolution: Unity and Diversity• HS-LS3-1: Heredity: Inheritance and Variation of Traits• HS-ETS1-3: Engineering Design• BS.03.01: Apply biotechnology principles, techniques, and processes to create transgenic species through genetic engineering.• BS.03.04: Apply biotechnology principles, techniques, and processes to enhance plant and animal care and production (e.g., selective breeding, pharmaceuticals, biodiversity, etc.).• PS.02.02: Apply principles of classification, plant anatomy, and plant physiology to plant production and management.
Learning Objectives: <p>By the end of the lesson, students will be able to:</p> <ol style="list-style-type: none">1. Extract DNA from food samples using InstaGene matrix and describe the role of each step in the process.2. Perform PCR to amplify specific DNA sequences and explain the purpose of plant and GMO primers.3. Conduct agarose gel electrophoresis to visualize PCR products and interpret band sizes to determine GMO status.4. Discuss the benefits and concerns of GMOs, evaluating their impact on agriculture, the environment, and human health.5. Analyze experimental controls (non-GMO and GMO-positive) to assess the validity of experimental results.
Length of Lesson: 180 min
Introduction/Background: <p>The world's growing population and shrinking arable land pose challenges to food production, prompting the development of genetically modified (GM) crops. These crops, engineered with genes like the <i>Bacillus thuringiensis</i> (Bt) toxin for pest resistance or herbicide resistance, aim to increase yields and reduce chemical use. However, GMOs spark debate over potential environmental risks (e.g., super-weeds, superbugs) and health concerns (e.g., allergies, antibiotic resistance). This lesson introduces students to biotechnology techniques—DNA extraction, PCR, and gel electrophoresis—used to detect GMO-specific DNA sequences in foods, fostering an understanding of genetic engineering and its societal implications.</p>
Key Concepts and Terms Covered: <ul style="list-style-type: none">• DNA extraction: Process of isolating DNA from cells.

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- **Polymerase Chain Reaction (PCR):** Technique to amplify specific DNA sequences.
- **Agarose gel electrophoresis:** Method to separate and visualize DNA fragments by size.
- **Genetically Modified Organisms (GMOs):** Organisms with altered DNA via genetic engineering.
- **Primers:** Short DNA sequences (plant primers, GMO primers) that initiate DNA replication in PCR.
- **InstaGene matrix:** Negatively charged beads that chelate metal ions to prevent DNA degradation.
- **Chloroplast gene:** Photosystem II gene common to plants, used as a control in PCR.
- **Bt toxin:** Protein from *Bacillus thuringiensis*, lethal to pests like European corn borers.
- **Molecular weight ruler:** DNA standard for sizing PCR products.
- **Thermal cycler:** Machine for rapid heating/cooling in PCR.
- **SYBR Safe:** Fluorescent DNA stain for gel visualization.
- **Biotechnology, genetic engineering, herbicide resistance, pest resistance, cross-pollination, super-weeds, superbugs.**

Materials:

- [Bio-Rad GMO Investigator™ kit](#)
- Test foods: tortilla chips (white/yellow and blue corn), corn muffin mix, golden yellow cake mix, fresh sweet corn, whole kernel golden corn, papaya, frozen edamame (in pod and shelled), veggie sausage links, strawberries
- Non-GMO control food (e.g., rolled oats)
- GMO-positive control DNA
- InstaGene matrix (500 µL per tube)
- Plant master mix (PMM, green primers) and GMO master mix (GMM, red primers)
- 3% agarose gels with SYBR Safe stain
- 1x TAE running buffer (1,500–2,000 mL)
- 1x Orange G loading dye
- PCR molecular weight ruler
- Equipment: water bath (95°C), microcentrifuge, balance, mortar and pestle, micropipets (2–20 µL), pipet tips (aerosol barrier), gel electrophoresis chambers, power supply, UV transilluminator, thermal cycler
- Common lab supplies: ice baths, marking pens, disposable transfer pipets, screwcap tubes, PCR tubes, microtube holders
- [Strawberry DNA extraction kit \(Carolina Biological Supply\)](#): 95% ethanol, DNA extraction buffer, cheesecloth, funnels, resealable plastic bags, 50-mL and 15-mL tubes, wooden sticks
- [GMO Test Worksheet](#), [Strawberry Extraction Guide](#), [Strawberry Extraction Worksheet](#) OR you can use this [worksheet](#).

Activities of the Session:

Attention Grabber:

Objective: Students will explore the concept of GMOs by designing their own genetically modified crops,10, addressing common questions and concerns about GMOs.

Materials

- Index cards or paper slips
- Markers, colored pencils, or crayons
- Handouts with basic GMO information (optional)
- Whiteboard or chart paper for group discussion

Steps

1. Introduction (10 minutes)

- Briefly explain what GMOs are: crops or organisms whose genetic material has been altered to introduce specific traits (e.g., pest resistance, drought tolerance).
- Discuss real-world examples (e.g., Bt corn, Golden Rice).
- Highlight pros (e.g., increased yield, reduced pesticide use) and cons (e.g., environmental concerns, public perception).
- Encourage students to ask questions to set the stage for the activity.

2. Design Your GMO Crop (20 minutes)

- Divide students into small groups (3-4 per group).
- Each group is tasked with creating a "new" GMO crop. Provide each group with an index card or paper to draw and describe their crop.
- Instructions:
 - Choose a base crop (e.g., tomato, rice, wheat).
 - Decide on one or two genetic modifications (e.g., glows in the dark, grows faster, resists drought). Encourage creativity but tie it to realistic genetic traits.
 - Draw the crop and label its modified traits.
 - Write a short description (2-3 sentences) explaining the modification, its benefits, and one potential concern.
- Example: A tomato modified to produce its own insect-repelling toxin, reducing pesticide use, but with concerns about crossbreeding with wild plants.

3. Present and Discuss (15 minutes)

- Each group presents their GMO crop to the class, explaining their design and its pros and cons.
- After each presentation, the class votes on whether they would "approve" the GMO for market (using raised hands or anonymous slips).
- Facilitate a brief discussion:
 - What makes this GMO useful or risky?
 - How might consumers react to it?
 - What testing would be needed before release?
- Write key points from the discussion on the board (e.g., ethical concerns, environmental impact, food security).

4. Reflection and Wrap-Up (10 minutes)

- Ask students to reflect:
 - What surprised them about GMOs?
 - How do they feel about GMOs in food?
 - What are the trade-offs of using GMOs?
- Summarize key takeaways: GMOs can address global challenges like hunger but require careful regulation and public trust.
- Optional: Reward creative or thoughtful contributions with small prizes or stickers.

Outcomes:

- Understand the basic science of GMOs.
- Evaluate the benefits and risks of genetic modification.
- Practice creative problem-solving and teamwork.
- Engage in critical thinking about science and society.

Session 1 (60 min): DNA Extraction and PCR Setup

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- **Teacher:** Prepare materials (aliquot InstaGene matrix, PMM, GMM, control DNA), set up ice baths, and demonstrate grinding technique. Divide students into groups and assign roles (non-GMO control, test foods 1–3, GMO-positive control).
- **Students:**
 1. Grind 0.5–2 g of non-GMO control and test foods with distilled water to form a slurry, add 50 μ L to InstaGene matrix tubes, boil at 95°C for 5 min, and centrifuge to isolate DNA.
 2. Set up 10 PCR tubes with 20 μ L PMM or GMM, add 20 μ L of respective DNA samples, centrifuge briefly, and place tubes in thermal cycler (program: 1 cycle at 94°C for 2 min, 40 cycles of 94°C/1 min, 59°C/1 min, 72°C/2 min, 1 cycle at 72°C for 10 min, hold at 12°C).
- **Discussion:** Answer lab report questions on DNA extraction (e.g., purpose of non-GMO control, organelle location of plant DNA).

Session 2 (60 min): Electrophoresis and Analysis

- **Teacher:** Prepare 3% agarose gels, set up electrophoresis chambers, and demonstrate gel loading. Provide UV safety instructions and image gels using Gel Logic 1500.
- **Students:**
 1. Add 10 μ L Orange G loading dye to PCR samples, practice loading dye in unused wells, and load 20 μ L of samples and molecular weight ruler into gel lanes.
 2. Run gels at 150 V for 20 min, visualize under UV transilluminator, and record band presence/absence in a table.
 3. Analyze results using the provided flowchart to determine GMO status of test foods.
- **Discussion:** Answer lab report questions on electrophoresis (e.g., purpose of molecular weight ruler, band separation).

Session 3 (Optional, 60 min): Strawberry DNA Extraction

- **Teacher:** Prepare strawberry DNA extraction materials (95% ethanol, extraction buffer, cheesecloth, etc.) and distribute to groups.
- **Students:** Mash strawberries, add extraction buffer, filter through cheesecloth, add ethanol to precipitate DNA, and spool DNA with a wooden stick. Discuss observations and complete the Strawberry DNA Extraction Review worksheet.

Engagement:

- **Interactive Lab Activities:** Hands-on DNA extraction, PCR setup, and gel electrophoresis keep students active and curious.
- **Group Discussion:** Divide students into two groups to debate GMO benefits (e.g., reduced pesticide use, higher yields) versus concerns (e.g., super-weeds, health risks), encouraging critical thinking and peer interaction.
- **Real-World Context:** Connect lab results to grocery store foods, prompting students to consider GMOs in their diets.
- **Visual Results:** Visualizing DNA bands on gels and spooling strawberry DNA provide tangible outcomes that excite students.
- **Role Assignment:** Assigning roles (e.g., non-GMO control, test food) fosters teamwork and responsibility.

Evaluation:

- **Formative Assessment:** Monitor student participation during lab activities and group discussions, ensuring accurate pipetting, sample handling, and engagement in GMO debate.
- **Lab Report:** Students complete questions from each activity, demonstrating understanding of DNA extraction, PCR, electrophoresis, and GMO analysis. The final table in Activity D evaluates their ability to interpret gel results and determine GMO status.

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- **Strawberry DNA Worksheet:** Optional activity assesses understanding of DNA extraction principles through written responses.
- **Rubric:** Evaluate lab reports based on accuracy (correct band sizes, GMO status), completeness (all questions answered), and critical thinking (e.g., explaining control validity).

Extensions and Modifications:

- **Advanced Learners:** Challenge students to research additional GMO detection methods (e.g., ELISA) or design primers for other GM traits.
- **Struggling Learners:** Provide pre-measured food samples and simplified instructions for pipetting. Pair with peers for support during complex steps like PCR setup.
- **Time Constraints:** Skip the optional strawberry DNA extraction or pre-grind test foods to save time.
- **Equipment Limitations:** If thermal cyclers or UV transilluminators are unavailable, use pre-amplified PCR products or virtual gel simulation software.
- **Diverse Learners:** Offer visual aids (e.g., labeled diagrams of PCR and electrophoresis) and translated instructions for non-English speakers.

Application:

This lesson connects to daily life by empowering students to test foods they consume for GMOs, helping them become smart consumers who make informed choices about their diet and agriculture. It highlights the importance of checking food labels, as some products may be labeled "GMO-free" for marketing, while others without the label could also be free of GMOs due to the absence of genetically modified varieties in certain crops (e.g., apples or wheat). Understanding biotechnology principles prepares students for careers in agriculture, food science, or genetic engineering. The debate on GMO benefits versus concerns encourages engagement with public policy and environmental issues, such as sustainable farming and food labeling laws, fostering critical thinking about consumer choices and regulatory transparency.

Resources:

- Bio-Rad GMO Investigator™ Kit Manual
- Carolina Biological Supply Company: Strawberry DNA Extraction Kit
- National Center for Biotechnology Information (NCBI): Resources on GMOs and biotechnology
- USDA: Information on genetically engineered crops
- Textbooks: *Campbell Biology* (Chapter on Biotechnology)
- Online videos: GMO debates and PCR tutorials

Laboratory

Genetically Modified Organisms Investigation

Objectives: Learn the principles, techniques, and applications of DNA extraction, PCR, and agarose gel electrophoresis.

This manual is modified from the instructions for Bio-Rad GMO Investigator™ kit.

TA PREPARATION:

1. Obtain the following items from a grocery store (by Dr. Lu):

Name	Maker	Size	Storage
Tortilla chips, white or yellow corn	On the border	18 oz	Room temperature
Tortilla chips, blue corn	On the border	7.25 oz	Room temperature
Corn muffin mix	JIFFY	8.5 oz	Room temperature
Golden yellow cake mix	JIFFY	8.5 oz	Room temperature
Fresh sweet corn on the cob	Meijer	Each	4°C
Whole kernel golden corn*	Meijer	16 oz	-20°C
Papaya (with skin removed)	SM Corporation	Each	4°C
Frozen edamame (soybean) in the pod	Meijer	16 oz	-20°C
Frozen shelled edamame	Pictsweet Farms	8 oz	-20°C
Veggie sausage links* (contain soybean)	Morningstar farms	8 oz	-20°C
Strawberries	Astin Farm	16 oz	4°C
*Frozen corns and links are difficult to grind. Place them at 4°C the day before the class.			

2. Fill four Styrofoam boxes (4019 Haenicke, 1 per group) with ice.

3. Set up items listed in the Bio-Rad GMO Investigator™ kit. Note that DNA extraction and PCR setup will be done on **June 27** and electrophoresis will be done on **July 3**. Aliquot InstaGene matrix, PMM, and GMM on **June 27**.

- Aliquot 3 sets of test foods (e.g., Tortilla chips, sweet corn, papaya, and soybean; 1 or 2 g each). •

Label 3 sets of four 1.5-mL screwcap tubes with “non-GMO”, “test 1”, “test 2”, and “test 3”. Place a bottle of InstaGene matrix that contains a stir bar on a stirrer plate, keep stirring so that the InstaGene beads are evenly distributed during aliquoting. Use a 1-mL big-mouth pipet tip to transfer 0.5 mL of newly resuspended InstaGene matrix to each 1.5-mL screwcap tube.

- Label three 0.5-mL centrifuge tubes with “GMO+ DNA”. Add 45 μ L of GMO-positive control DNA each.
- Label two 1.5-mL centrifuge tubes with “PMM” or “GMM”. Add 340 μ L of clear 2x PCR master mix to each tube. Add 6.8 μ L of green plant primers to the “PMM” tube, add 6.8 μ L of red GMO primers to the “GMM” tube, shake vigorously to mix, spin down briefly, store on ice.
- Label 3 sets of 10 PCR tubes with numbers 1-10. Add 20 μ L of PMM to PCR tubes 1, 3, 5, 7, and 9. Add 20 μ L of GMM to PCR tubes 2, 4, 6, 8, and 10.

4. Bring a UV transilluminator from 4010 Haenicke to 1120 Wood on **July 3**. Make three agarose gels with SYBR safe fluorescent DNA gel stain in 4019 Haenicke, bring three sets of gel apparatus, power supply, and 1x Orange G loading dye (for gel loading practice only) to 1120 Wood on **July 3**.

- Recipe for 10-cm agarose gel: two 20-well combs total on a 10-cm-long tray; 120 mL 1x TAE, 3.6 g agarose, 2-3 min microwave until every piece of agarose is dissolved, shake after every minute; cool down to 50°C in a water bath for ~3 min; add 12 μ L of SYBR Safe DNA Gel Stain into the gel mix, mix well and gently pour into the tray; let solidify for 25 min.

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- Recipe for 7-cm agarose gel: one 20-well comb on a 7-cm-long tray; 84 mL 1x TAE, 2.52 g agarose, 1-2 min microwave until every piece of agarose is dissolved; see above for other steps.
- Label two 1.5-mL microcentrifuge tubes with “PCR MW Ruler”, add 64 μ L of clear PCR MW Ruler and 16 μ L of (5x) Orange G loading dye in each tube, mix by pipetting up and down, spin down briefly. Split into four 1.5-mL microcentrifuge tubes and label them.
- Label three 1.5-mL microcentrifuge tubes with “1x Orange G”, add 288 μ L of nuclease-free H₂O and 72 μ L of (5x) Orange G loading dye in each tube, mix by pipetting up and down, spin down briefly.

Introduction

With the world population exploding and farmable land disappearing, agricultural specialists are concerned about the world's ability to produce enough food to feed the growing population. Environmentalists are concerned about the overuse of pesticides and herbicides and the long-term effects of these chemicals on the environment and human health. Might there be a solution to both of these problems? The biotechnology industry thinks so. Its proponents believe genetically modified organisms (GMOs), particularly genetically modified (GM) crop plants, can solve both problems. This proposed solution, however, has met with great opposition throughout the world. Dubbed "frankenfoods" by opponents and restricted in most European countries, GMOs are widely produced and sold in the United States. Currently in the US, foods that contain GMOs do not have to be labeled as such.

Genetic manipulation of crop plants is not new. Farmers have been genetically modifying crops for centuries. Crop breeding to encourage specific traits, such as high yield, is still an important part of agriculture today. However, there is now the option to place genes for selected traits directly into crop plants. These genes do not have to originate from the same plant species—in fact, they do not have to come from plants at all. One popular class of GM crops has a gene from the soil bacterium *Bacillus thuringiensis* (Bt) inserted into their genomes. Bt crops produce a protein called delta-endotoxin that is lethal to European corn borers, a common pest on corn plants. Farmers who plant Bt crops do not have to apply pesticide because the plants produce the toxic protein

inside their cells. When the corn borers feed on the genetically modified plant, they die. Other GMOs include those that are herbicide-resistant delayed for fruit ripening, are resistant to fungi or drought, have increased crop yield, or bear improved fruits.

Many people object to the use of GM crop plants. They argue that there is a potential to create super-weeds through cross-pollination with herbicide-resistant crops or that superbugs will evolve that are no longer resistant to the toxins in pest-resistant crops. Many are concerned with potential allergic reactions to the novel proteins or antibiotic resistance arising from the selectable markers used to develop the crops or other unforeseen effects on public health. Proponents of GM foods argue these crops are actually better for the environment. Fewer toxic chemicals are put into the environment and thus fewer toxic chemicals can harm the environment and human health. In addition, these crops can preserve arable land by reducing stresses on the land, improve the nutritional value of food in developing countries, and allow crops to be grown on previously unfarmable land.

Benefits and Concerns of GM Crops

After watching the videos, the TA will divide students into two subgroups. One group will identify and discuss at least five benefits of GM crops and the other group will identify and discuss at least five concerns of growing GM crops. The two groups will then discuss whether the benefits of GM crops outweigh the concerns of growing GM crops.

Experiment Overview

Whatever position one takes in the GMO debate, it would be beneficial to be able to test foods found in the grocery store for the presence of GMO-derived products. This can be done in several ways. One would be to use an antibody-based test such as the enzyme-linked immunosorbent assay (ELISA), which can detect the proteins that are produced specifically by GM crops. However, the ELISA is not useful for testing foods that have been

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highly processed, because the proteins have most likely been destroyed and different GM foods produce different proteins. Another method is to use the polymerase chain reaction (PCR) to look for a DNA sequence common to GM foods. DNA is more resistant than proteins to processing and can be extracted from even highly processed foods. It is these GMO DNA sequences that we will be testing for in this laboratory.

In the first activity you will extract genomic DNA from food samples, in the second lab you will run PCR reactions to amplify GMO and natural plant sequences from the DNA, and in the third lab you will electrophorese the amplified samples to visualize the DNA.

Let's see if your favorite food contains GMOs!

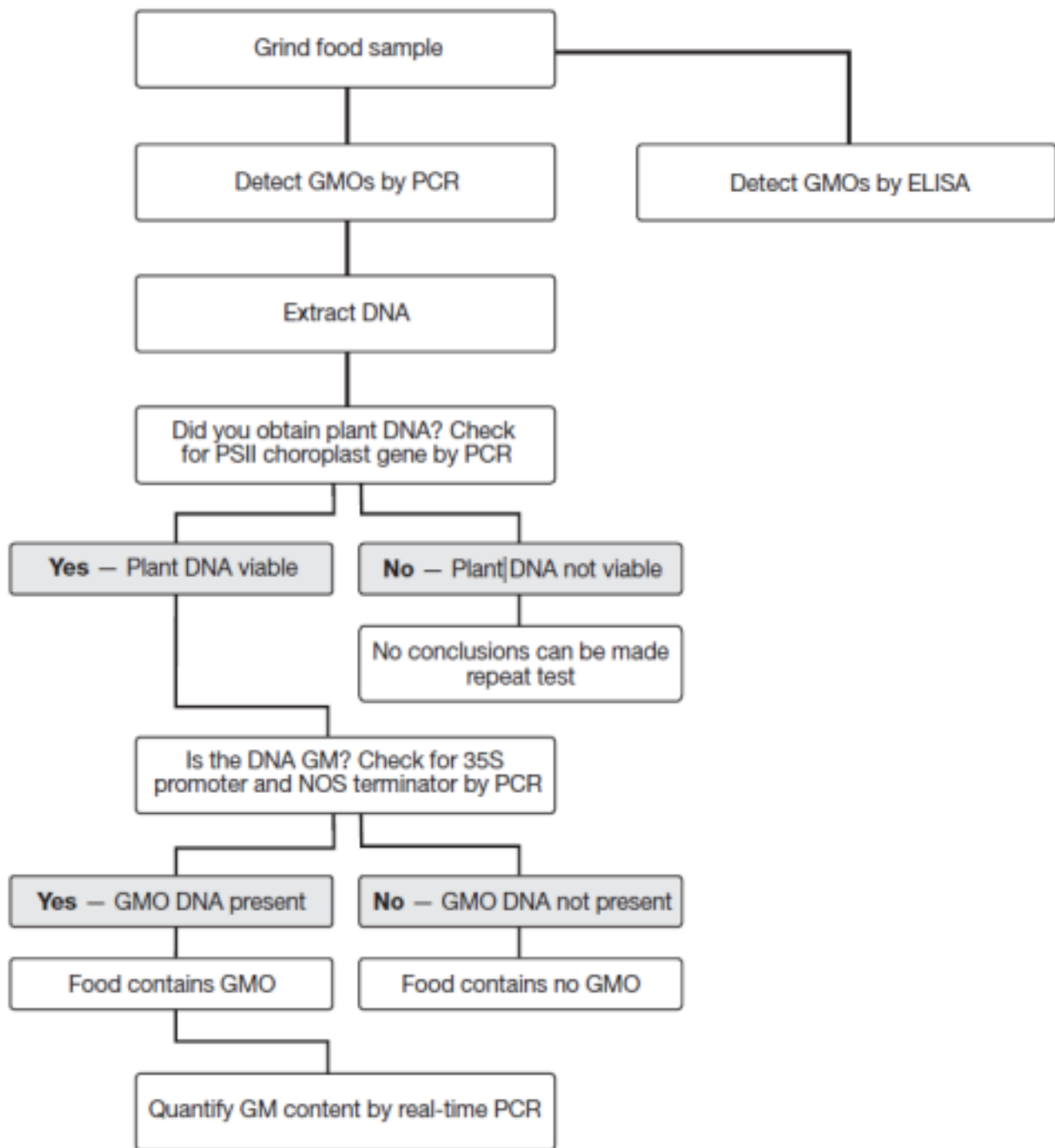


Figure 1. How to detect GMOs in food.

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Figure 2. Detecting GM foods by PCR. Genomic DNA is extracted from test foods (Lesson 1) and then two PCR reactions are performed on each test food genomic DNA sample (Lesson 2). One PCR reaction uses primers specific to a common plant gene (plant primers) to verify that viable DNA was successfully extracted from the food. No matter whether the food is GM or not, this PCR reaction should always amplify DNA (See lanes 1 and 3 of the gel above). The other PCR reaction uses primers specific to sequences commonly found in GM crops (GMO primers). This PCR reaction will only amplify DNA if the test food is GM (See lane 4). If the test food is non-GM, then the GMO primers will not be complementary to any sequence within the test food genomic DNA and will not anneal, so no DNA will be amplified (see lane 2). To find out whether DNA has been amplified or not, the PCR products are electrophoresed on a gel and stained to visualize DNA as bands (Lesson 3). A molecular weight ruler (lane 5) is electrophoresed with the samples to allow the sizes of the DNA bands to be determined.

Student Activity A: Extraction of DNA From Food Samples

You will extract DNA from a control non-GMO food and a grocery store food item that you will test for the presence of GMOs. The most commonly modified foods are corn and soy-based, and so the test food could be fresh corn or soybeans, or a prepared or processed food such as cornmeal, cheese puffs, veggie sausage, etc. You will process the non-GMO control first.

You will first weigh your food sample, then grind it with water to make a slurry. You will then add a tiny amount of the slurry to a screwcap tube containing InstaGene matrix and boil it for 5 min.

The cellular contents you are releasing from the ground-up sample contain enzymes (DNases) that can degrade the DNA you are attempting to extract. The InstaGene matrix is made of negatively charged microscopic beads that “chelate” or grab metal ions out of solution. It chelates metal ions such as Mg^{2+} , which is required as a cofactor in enzymatic reactions. When DNA is released from your sample in the presence of the InstaGene matrix, the charged beads grab the Mg^{2+} and make it unavailable to the enzymes that would degrade the DNA you are trying to extract. This allows you to extract DNA without degradation. Boiling the samples destroys these enzymes.

After you centrifuge the samples to remove the InstaGene matrix and debris, the supernatant will contain intact extracted DNA. This extracted DNA will be used in the next laboratory as your target DNA.

Student Workstation

Material Quantity

Ice bath 1
Screwcap tube with 500 μ L InstaGene matrix 4
Beaker of distilled water 1
Food samples 4
Disposable plastic transfer pipets (DPTP) 4
2–20 μ L micropipet (if preparing non-GMO food control) 1
2–20 μ L pipet tips, aerosol barrier 1 rack
Mortar and pestle 4
Marking pen 1



Common Workstation

Material Quantity

Water bath or dry bath set to 95°C 1
Microfuge or mini centrifuges 1
Balance and weigh boats 1



Methods

Decide among your group who is in charge of non-GMO food control, test food 1, test food 2, test food 3, and GMO-positive control DNA. Note that GMO-positive control DNA has already been extracted. Therefore, no grinding or extraction is needed for this DNA sample.

Grind non-GMO food control (your TA may perform this step for you)

1. Find four 1.5-mL screwcap tubes containing 500 μL of InstaGene matrix. They are labeled as: non-GMO, test 1, test 2, and test 3.

2. Place 0.5 - 2 g of certified non-GMO food control (i.e., rolled oats) in mortar. Grind with pestle for ~2 min so that non-GMO food control is broken into small pieces.

2. Use a brand-new transfer pipet, add 5 mL of distilled water for every gram of food using the graduations on the transfer pipet. To calculate the volume of water you need, multiply the mass in grams of the food weighed out by 5 and add that many millimeters.

Mass of Food = ____ g \times 5 = ____ mL

3. Grind with pestle for at least 2 min until a slurry is formed.

4. Add 5 volumes of water again (i.e., another 5 mL to 1 g of food) and mix or grind further with pestle until the slurry is smooth enough to pipet.

5. Add **50 μL** of ground slurry to the screwcap tube containing 500 μL of InstaGene matrix labeled “non-GMO” using the recently used transfer pipet.

6. Recap tube and shake well.

7. Place the mortar and pestle in a wash bin. Do not reuse the mortar and pestle until they are washed, rinsed, dried, and autoclaved.



Grind Test Food

1. Place 0.5 - 2 g of test food 1 in a mortar. Grind with pestle for ~2 min so that the test food is broken into small pieces.

2. Use a brand-new transfer pipet, add 5 mL of distilled water for every gram of food using the graduations on the transfer pipet. To calculate the volume of water you need, multiply the mass in grams of the food weighed out by 5 and add that many millimeters.

Mass of food = ____ g x 5 = ____ mL

3. Grind with pestle for at least 2 min until a slurry is formed.

4. Add 5 more volumes of water (i.e., another 5 mL to 1 g of food) and mix or grind further with pestle until the slurry is smooth enough to pipet.

5. Add **50 μ L** of ground slurry to the screwcap tube labeled “Test 1” using the 50 μ L mark on the recently used transfer pipet.

6. Recap tube and shake well.

7. Place the mortar and pestle in a wash bin. Do not reuse the mortar and pestle until they are washed, rinsed, dried, and autoclaved.

Extract DNA



8. Repeat steps 1- 7 for test food 2

and 3. **Process Samples to**

1. Place non-GMO food control and test food 1-3 sample tubes, which contain InstaGene Matrix, in a 95°C water bath or dry bath for 5 min.

2. Place tubes in a centrifuge in a balanced conformation and spin for 5 min at max speed.

3. Store tubes in on ice.

Questions for Discussion in the Lab Report:

1. How can you test a food to find out if it contains material derived from a genetically modified organism (GMO)?

*Hint: Answers could be found under **Experiment Overview**.*

2. In what organelles is plant DNA located?

3. Many foods containing GM crops are highly processed. Can you suggest how DNA from whole plants may differ from that extracted from processed foods, e.g., corn chips, cornmeal, etc.?

4. What molecules are present in the cell that might interfere with DNA extraction?

5. Why did we also perform analysis on food that is known to be a non-GMO food control?

Why was the non-GMO food control prepared prior to the test food samples?

Student Activity B: Set Up PCR Reactions

In the last laboratory, you extracted DNA from a certified non-GMO food sample and a test food sample that you are analyzing for the presence of GMO DNA sequences. In this lab you will prepare those two samples and a positive control (GMO-positive template DNA) for the polymerase chain reaction (PCR).

PCR is DNA replication in a test tube. PCR allows you to amplify specific sections of DNA and make millions of copies of the target sequence. Your experiment is to determine whether or not the DNA you extracted from food in Lesson 1 contains or does not contain the target sequences of interest typically found in genetically modified (GM) foods.

PCR Review

PCR is such a powerful tool because of its simplicity and specificity. All that is required are minute quantities of the DNA template you want to amplify, DNA polymerase, two DNA primers, four DNA base pair subunits and buffers.

Because PCR identifies a specific sequence of DNA and makes millions of copies of (or amplifies) that sequence, it is one of the most useful tools of molecular biology. Scientists use PCR to obtain the large amounts of a specific sequence of DNA that are necessary for such techniques as gene cloning, where DNA is physically moved from one genome to another. You are using the property of PCR that allows identification of a specific sequence, namely, the ability of PCR to search out a single sequence of a few hundred base pairs in a background of billions of base pairs. For example, the corn genome has 2.5 billion base pairs of DNA. This sequence is then amplified so that there are millions of copies of it so that it stands out from the few copies of the original template DNA.

PCR locates specific DNA sequences using primers that are complementary to the DNA template. Primers are short strands of DNA (usually between 6 and 30 base pairs long) called oligonucleotides. Two primers are needed to amplify a sequence of DNA, a forward primer and a reverse primer. The two primers are designed and synthesized with a specific sequence of nucleotides such that they can anneal at opposite ends of the target DNA sequence on the complementary strands of the target DNA template. The target DNA sequence is copied by the DNA polymerase reading the complementary strand of template DNA and adding nucleotides to the 3' ends of the primers (see Fig 2). Primers give the specificity to the PCR, but they are also necessary because DNA polymerase can only add nucleotides to double-stranded DNA.

During PCR, double-stranded DNA template is separated by heating it, then each primer binds (anneals) to its complementary sequence on each of the separated DNA strands, and DNA polymerase elongates each primer by adding nucleotides to make a new double-stranded DNA (see fig 2).

The DNA polymerase used in PCR must be a thermally stable enzyme because the PCR reaction is heated to 94°C, which would destroy the biological activity of most enzymes. The most commonly used thermostable DNA polymerase is Taq DNA polymerase. This was isolated from a thermophilic bacterium, *Thermus aquaticus*, which lives in high temperature steam vents such as those in Yellowstone National Park.

PCR Step by Step

PCR has three steps, a denaturing step, an annealing step, and an elongation step (**Figures 3** and the table below). During the denaturing step, the DNA template is heated to 94°C to separate (or denature) the double-stranded DNA molecule into two single strands. The DNA is then cooled to 59°C to allow the primers to locate and anneal (bind) to the DNA. Because the primers are so much shorter than the template DNA, they will anneal much more quickly than the long template DNA strands at this temperature. The final step is to increase the temperature of the PCR reaction to 72°C, which is the optimal temperature for the DNA polymerase to function. In this step the DNA polymerase adds nucleotides (A, T, G, or a C) one at a time at the 3' end of the primer to create a

complementary copy of the original DNA template. These three steps form one cycle of PCR. A complete PCR amplification undergoes multiple cycles of PCR, in this case 40 cycles.

Figure 3. A complete cycle of PCR.

The entire 40 cycle reaction is carried out in a test tube that has been placed in a thermal cycler or PCR machine. This is a machine that contains an aluminum block that can be rapidly heated and cooled. The rapid heating and cooling of this thermal block is known as thermal cycling.

Two new template strands are created from the original double-stranded template during each complete cycle of PCR. This causes exponential growth of the number of target DNA molecules, i.e., the number of target DNA molecules doubles at each cycle; this is why it is called a chain reaction. Therefore, after 40 cycles there will be 240, or over 1,100,000,000,000 times more copies than at the beginning. Once the target DNA sequences of interest have been sufficiently amplified, they can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the PCR products of interest.

Step	Function	Number of Temperature	Duration	Cycles
Initial denaturation	Denature	94°C	2 min	1
PCR amplification	Denature	94°C	1 min	40 1 min
	Anneal	59°C		
	Extend	72°C	2 min	
Final extension	Extend	72°C	10 min	1
*Hold	Hold	4°C	Indefinite	1

Your Task for This Activity

For this experiment you will set up two PCR reactions for each DNA sample, which makes 6 PCR reactions in total. One PCR reaction, using the plant master mix (PMM), is a control to determine whether or not you have successfully extracted plant DNA from your test food. This is done by identifying a DNA sequence that is common to all plants by using primers (**colored green** in the kit) that specifically amplify a section of a chloroplast gene used in the light reaction (photosystem II). Why is this necessary? What if you do not amplify DNA using the GMO primers? Can you conclude that your test food is not GM or might it just be that your DNA extraction was unsuccessful? If you amplify DNA using the plant primers, you can conclude that you successfully amplified DNA, therefore whether or not you amplify DNA with your GMO primers, you will have more confidence in the validity of your result.

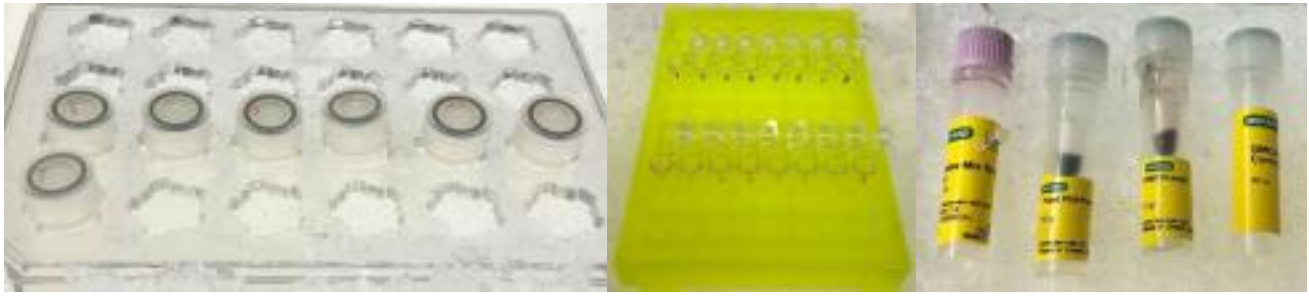
The second PCR reaction you carry out will determine whether or not your DNA sample contains GM DNA sequences. This is done by identifying DNA sequences that are common to most (~85%) of all GM plants using primers specific to these sequences. These primers are **colored red/purple** and are in the GMO master mix (GMM).

Why do you have to set up a PCR reaction with DNA from certified non-GMO food? What if some GMO-positive DNA got into the InstaGene or master mix from a dirty pipet tip or a previous class? This DNA could be amplified in your test food PCR reaction and give you a false result. By having a known non-GMO control that you know should not amplify the GMO target sequences, you can tell if your PCR reactions have been contaminated by GMO-positive DNA.

Student Workstations

Material Quantity

Ice bath 1
 5 PCR tubes with 20 µL of GMO master mix (red/purple) (on ice) 1
 5 PCR tubes with 20 µL of Plant master mix (green) (on ice) 1
 GMO-positive control DNA (on ice) 1
 Test food 1-3 DNA (from previous lab) 3
 Non-GMO food control DNA (from previous lab) 1
 PCR tube adaptors (optional) 10
 Microtube holder 1
 Marking pen 1
 2–20 µL adjustable-volume micropipet 1
 2–20 µL pipet tips, aerosol barrier 1 rack



1. Find ten PCR tubes labeled with numbers 1 - 10. The numbers correspond to the following tube contents: **Tube # DNA Master Mix**

- | | | |
|----|-------------------------------------|-------------------------------------|
| 1 | 20 μ L Non-GMO food control DNA | 20 μ L Plant master mix (green) |
| 2 | 20 μ L Non-GMO food control DNA | 20 μ L GMO master mix (red) |
| 3 | 20 μ L Test food 1 DNA | 20 μ L Plant master mix (green) |
| 4 | 20 μ L Test food 1 DNA | 20 μ L GMO master mix (red) |
| 5 | 20 μ L GMO-positive control DNA | 20 μ L Plant master mix (green) |
| 6 | 20 μ L GMO-positive control DNA | 20 μ L GMO master mix (red) |
| 7 | 20 μ L Test food 2 DNA | 20 μ L Plant master mix (green) |
| 8 | 20 μ L Test food 2 DNA | 20 μ L GMO master mix (red) |
| 9 | 20 μ L Test food 3 DNA | 20 μ L Plant master mix (green) |
| 10 | 20 μ L Test food 3 DNA | 20 μ L GMO master mix (red) |

2. Keep the PCR tubes on ice for the remaining steps.

3. Note that PCR tubes 1, 3, 5, 7, and 9 contain 20 μ L of green plant master mix (PMM) and PCR tubes 2, 4, 6, 8, and 10 contain 20 μ L of red GMO master mix (GMM).

4. Using a fresh pipet tip for each PCR tube, add 20 μ L of the DNA to each PCR tube as indicated in the table above. Take care not to transfer any of the InstaGene beads to your PCR reaction. If the beads are disrupted, re-centrifuge your DNA samples to pellet the beads.

- Add 20 μ L of non-GMO food control DNA to PCR tube 1 and pipet up and down to mix. Discard your tip.
Recap tubes.
- Use a fresh tip to add 20 μ L of non-GMO food control DNA to PCR tube 2 and mix. Discard your tip.
Recap tubes.
- Similarly add 20 μ L of test food DNA to PCR tubes 3-4 and 7-10, changing your tip for every tube. Recap tubes.
- Similarly add 20 μ L of GMO-positive control DNA to PCR tubes 5-6, changing your tip for every tube.
Recap tubes.

5. Centrifuge your PCR tubes on a mini centrifuge for a few seconds.

6. When instructed to, place the PCR tubes in a thermal cycler in 4019 Haenicke Hall, use the PCR program called "GMO". The hold temperature is changed from 4°C to 12°C to extend the lifetime of the thermo cycler. 1

cycle: 94°C 2 min

40 cycles: 94°C 1 min

59°C 1 min

72°C 2 min

1 cycle: 72°C 10 min

1 cycle: 12°C indefinite

Questions for Discussion in the Lab Report:

1. What chemicals and molecules are needed for PCR, and what is the function of each component?
2. Why do we perform two PCR reactions on each DNA sample?
3. What is the purpose of the GMO-positive control DNA?

Student Activity C: Electrophoresis of PCR Products

You have completed your PCR amplification. You cannot, however, at this point determine whether or not you have PCR products. To do this, you must visualize your products. You will do this using gel electrophoresis.

Your PCR product bands are very small compared to those in other DNA experiments you may have done. For example, fragments produced from a HindIII digest of lambda DNA are 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, and 500 base pairs (bp). The product band sizes in this lab are 455 bp for the plant primers and 200 bp for the

GMO primers, and a 1% gel would not separate these bands. Instead, a tighter gel matrix is needed to impede the movement of these bands so that they are separated more on the gel and can be seen. Therefore, if you are using agarose electrophoresis, you will use a 3% agarose gel.

Regardless of the gel type, you will load a molecular weight ruler (DNA standard) so that you have a reference to determine your product bands' sizes. The gel will then be stained with Fast Blast stain to make the bands visible.

Student Workstation

Material **Quantity** Gel (3% agarose, 1x TAE, 0.01% SYBR Safe) 1

Samples from previous lab period 10

Running buffer (1x TAE for agarose gels) 1,500–2,000 mL 1x Orange G loading dye 1 vial

PCR molecular weight ruler 1 vial

2–20 µL adjustable-volume pipet 1

1–20 µL pipet tips, aerosol barrier 1 rack

Gel electrophoresis chamber (may be shared by 2 workstations) 1

Power supply (may be shared by multiple workstations) 1

A UV transilluminator (at common workstation) 1

Methods

1. Set up your gel electrophoresis apparatus as instructed. Details on setting up electrophoresis equipment can be found in the Instructor's guide.

2. Using a fresh tip each time, add 10 µL of (5x) Orange G loading dye to each sample and mix well. 3a. If you are not confident about gel loading, you may practice by loading 20 µL of 1x Orange G loading dye to an unused well/lane (e.g., lane 12, 13, 14, 15, 16, 17, 18, 19, or 20).

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3b. Load 20 µL of the PCR molecular mass ruler and 20 µL of each sample onto your gel in the order indicated below.

Lane	Sample	Load volume
1	Sample 1: Non-GMO food control with green plant primers	20 µL
2	Sample 2: Non-GMO food control with red GMO primers	20 µL
3	Sample 3: Test food 1 with green plant primers	20 µL

4	Sample 4: Test food 1 with red GMO primers	20 µL
5	Sample 5: GMO-positive DNA with green plant primers	20 µL
6	Sample 6: GMO-positive DNA with red GMO primers	20 µL
7	PCR molecular weight ruler	20 µL
8	Sample 7: Test food 2 with green plant primers	20 µL
9	Sample 8: Test food 2 with red GMO primers	20 µL
10	Sample 9: Test food 3 with green plant primers	20 µL
11	Sample 10: Test food 3 with red GMO primers	20 µL

4. The run time and voltage will depend on the type of gel you are running. Run an agarose gel at 150 V for 20 min. Do not let the orange dye front migrate out of the agarose gel.

5. Visualize and image the gel on a UV transilluminator from above the UV blocking cover while wearing UV safe glasses. Have your TA image the gel with the Gel Logic 1500 imaging system in 2019 Haenicke and obtain the jpg image file from your TA.

Questions for Discussion in the Lab Report:

1. Why did you resolve your PCR products by electrophoresis?
2. Explain why DNA fragments separate according to size in an electrophoresis gel.
3. Why do you need a molecular weight ruler alongside your samples?

The diagram and the table below show some hypothetical agarose gel images.

Results for GMO-positive food Results for non-GMO food

PCR Sample	Band Size	Lane 1: Non-GMO food with green plant primers
Lane 1: Non-GMO food with green plant primers	455 bp	
Lane 2: Non-GMO food with red GMO primers	No band	
Lane 3: Test food 1 with green plant primers	455 bp	
Lane 4: Test food 1 with red GMO primers	200 bp or no band	
Lane 5: GMO-positive template with green plant primers	455 bp	
Lane 6: GMO-positive template with red GMO primers	200 bp	
Lane 7: PCR molecular weight ruler	1,000, 700, 500, 200, 100 bp	
Lane 8: Test food 2 with green plant primers	455 bp	
Lane 9: Test food 2 with red GMO primers	200 bp or no band	
Lane 10: Test food 3 with green plant primers	455 bp	
Lane 11: Test food 3 with red GMO primers	200 bp or no band	

The presence or absence of a 200 bp band in **lane 4** above indicates whether or not the test food contains GMOs. However, the validity of this result depends on the results from the other PCR reactions. The plant primers determine whether plant DNA was successfully extracted from the sample. The non-GMO food control is an indicator of false positive results, should they occur. If the non-GMO food control comes out as GMO-positive (showing a band in lane 2) it means that the PCR was contaminated at some point during processing. If your test food is also GMO-positive, you cannot trust this result. The GMO-positive template control is an indicator of false negatives. If the GMO-positive template control does not amplify, there is a problem with the PCR reaction and you cannot trust a GMO-negative result from your test food. The flow chart (**Figure 4**) on the next page shows how to evaluate these controls in a step-by-step manner.

Figure 4. Step-by step guide to analysis of results in the hypothetical agarose gel image. There is a typo in Step 3. The correct question should be: is there a 455 bp band from “non-GMO food” and plant primers (lane 1)?

According to the actual agarose gel image, indicate the presence or absence of bands in PCR reactions, the band sizes, and the GMO status of each test food in the table below and include the table in your lab report.

<i>Lane Label</i>	<i>Actual food item tested</i>	<i>Primers used</i>	<i>Band?</i>	<i>Band Size</i>	<i>GMO status according to our test</i>
1	<i>Non-GMO food control</i>	<i>Plant primers</i>	<i>Yes</i>	<i>455 bp</i>	<i>Non-GMO</i>
2		<i>GMO primers</i>	<i>No</i>	<i>Not relevant</i>	
3	<i>Test food 1</i> (_____)	<i>Plant primers</i>			
4		<i>GMO primers</i>			
5	<i>GMO-positive template</i>	<i>Plant primers</i>	<i>Yes</i>	<i>455 bp</i>	<i>GMO</i>
6		<i>GMO primers</i>	<i>Yes</i>	<i>200 bp</i>	
7	<i>PCR molecular weight ruler</i>	<i>NA</i>	<i>Yes</i>	<i>1,000, 700, 500, 200, 100 bp</i>	
8	<i>Test food 2</i> (_____)	<i>Plant primers</i>			
9		<i>GMO primers</i>			
10	<i>Test food 3</i> (_____)	<i>Plant primers</i>			
11		<i>GMO primers</i>			

Student Activity D Questions for Discussion in the Lab Report:

1. What were our actual test food items?
2. Did each of our test food items generate a 200 bp band with GMO primers?
3. What does this tell you about the GMO status of these test food items?
4. What other information do we need to confirm the GMO status of each food DNA sample?
5. How do the results of the other five PCR reactions help support or undermine the result for our test food items?

Hint: The other five PCR reactions are:

Non-GMO food with plant primers,

Non-GMO food with GMO primers,

Test food with plant primers,

GMO-positive template with plant primers, and

GMO-positive template with GMO primers.

6. If you were to repeat the procedure what laboratory practice might yield better results?

Strawberry DNA Extraction (Optional; no lab report required)

During the second part of the GMO laboratory, the students may extract DNA from fresh strawberries. The kit was ordered from Carolina Biological Supply Company.

TA preparation before the class:

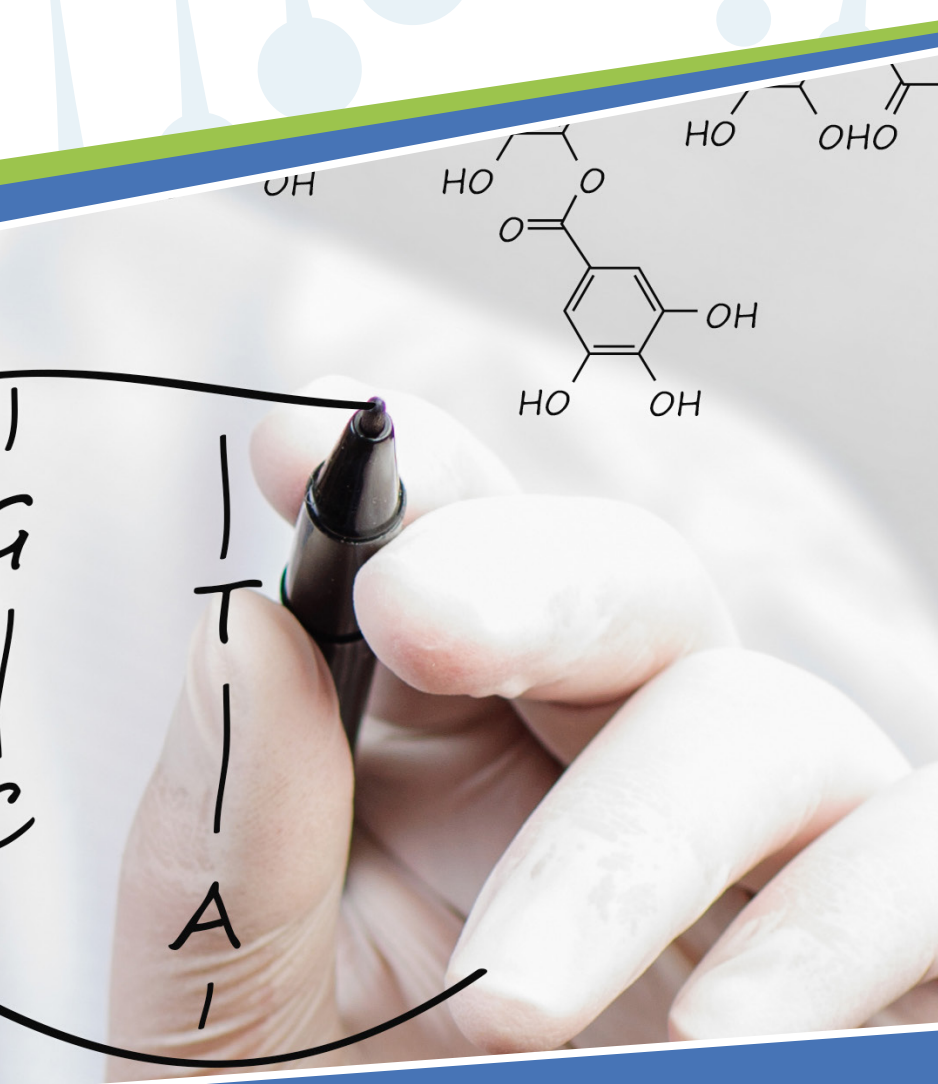
1. Chill the 95% ethanol by storing it in a freezer (preferred), refrigerator, or on ice until use.
2. If using frozen strawberries, allow the berries to thaw at room temperature before use. Do not heat the strawberries to hasten thawing, as this will hinder the DNA extraction process.
3. Prepare 400 mL of DNA extraction buffer. Use one of the 50-mL tubes (included) to measure 20 mL of detergent (included). Pour the detergent into a container along with 380 mL of water and the entire contents (10 g) of the salt pack (included). Stir to mix.
4. Aliquot 10 mL of extraction buffer into three or four 15-mL tubes (one tube/group).
5. Prepare three or four 5-mL aliquots of 95% ethanol in transfer vessels (one aliquot/group). An extra 15-mL tube is included for measuring out the 5-mL amounts.
6. Unroll cheesecloth from the spool and cut three or four pieces approximately 6" long. (The spool is about 8" wide, so each piece should measure about 6x8").
7. Reproduce the *Strawberry DNA Extraction Student Instructions* for each group.
8. For each group, distribute the following materials:
 - 10-mL aliquot of DNA extraction buffer in 15-mL tube
 - 5-mL aliquot of 95% ethanol in transfer vessel
 - 1 resealable plastic bag
 - 1 strawberry
 - 1 50-mL tube
 - 1 15-mL tube
 - 1 piece of cheesecloth
 - 1 funnel
 - 1 transfer pipet
 - 1 wooden stick

Strawberry DNA Extraction Student Instructions

1. Obtain one fresh or one frozen and thawed strawberry. If you are using a fresh strawberry, remove the green sepals (tops) from the berry.
2. Place the strawberry in a resealable bag.
3. Close the bag slowly, pushing all of the air out of the bag as you seal it.
4. Being careful not to break the bag, thoroughly mash the strawberry with your hands for two minutes.
5. Pour the 10-mL aliquot of DNA extraction buffer into the bag with the mashed strawberry. Reseal the bag.
6. Mash the strawberry for one additional minute.
7. Place a funnel into a 50-mL centrifuge tube. Fold the cheesecloth in half along the longer side and place it in the funnel to create a filter. The cheesecloth with overlap the edge of the funnel.
8. Pour the strawberry mixture into the funnel, filtering the contents through the cheesecloth and into the 50-mL centrifuge tube.
9. Pour 2 mL of the filtered contents from the 50-mL tube into a clean 15-mL tube. Use the lines on the side of the 15-mL tube to help measure the amount added.
10. Hold the 15-mL tube at an angle. Using a transfer pipet, carefully add 5 mL of cold 95% ethanol by running it down the inside of the tube. Add the 95% ethanol until the total volume is 7 mL (use the lines on the side of the tube to help you measure). You should have two distinct layers.
- Caution:** Do not mix the strawberry extract and the ethanol!
11. Watch closely as translucent strands of DNA begin to clump together where the ethanol layer meets the strawberry extract layer. Tiny bubbles in the ethanol layer will appear where the DNA precipitates.
12. Slowly and carefully rotate the wooden stick in the ethanol directly above the extract layer to wind (or "spool") the DNA. Remove the wooden stick from the tube and observe the DNA.

After the experiment, the TA will review the Questions for Discussion with the students. The TA will also photocopy and distribute the Strawberry DNA Extraction Review worksheet and have students answer the questions individually.

Strawberry DNA Extraction



TEACHER AND STUDENT MANUAL

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Photocopy the Student Guide as needed for use in your classroom.

This kit has been developed in collaboration with the DNA Learning Center, Cold Spring Harbor Laboratory. The activities have been tested by teachers and students participating in DNALC hands-on workshops.

Strawberry DNA Extraction

Overview

This kit provides teachers with the information, instructions, and materials needed to introduce middle school students to the concept and process of plant DNA extraction. Students learn what DNA is, how it is structured and contained within cells, and how it can be removed from cells. During the Prelab, students employ a castle analogy to understand both the structure and function of some plant cell components, as well as the techniques used to extract and observe DNA from plant cells.

During the Laboratory Investigation, students use hands-on scientific procedures to break through the barriers of a plant cell and isolate and observe DNA extracted from strawberry fruit. Assessment questions and Extension Activities are included, as is a Glossary of Important Terms. This kit accommodates up to 32 students working in pairs.

Correlation to the Next Generation Science Standards*

The activities in this kit address the following dimensions of the Next Generation Science Standards:

MS-LS1-2. Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function.

Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Developing and Using Models <ul style="list-style-type: none"> Develop and use a model to describe phenomena. 	LS1.A: Structure and Function <ul style="list-style-type: none"> Within cells, special structures are responsible for particular functions, and the cell membrane forms the boundary that controls what enters and leaves the cell. 	Structure and Function <ul style="list-style-type: none"> Complex and microscopic structures and systems can be visualized, modeled, and used to describe how their function depends on the relationships among its parts, therefore complex natural structures/systems can be analyzed to determine how they function.

To view additional national and local standards met by this kit, visit www.carolina.com/correlations.

*"Next Generation Science Standards" is a registered trademark of WestEd. Neither WestEd nor the lead states and partners that developed the Next Generation Science Standards were involved in the production of this product, and do not endorse it. **Source:** NGSS Lead States, 2013. *Next Generation Science Standards: For States, By States*. Retrieved from www.nextgenscience.org or ngss.nsta.org.



Activate Your Digital Resources

1. Visit CarolinaScienceOnline.com/activate
2. Log in or create an account
3. Enter or copy and paste code(s) received via “Your Carolina Digital Resources” email

Need help? Call Customer Service at 800.334.5551
or email csupport@carolina.com

What Teachers Are Saying

The digital access was amazing!!!

Biology Teacher, NC

I love the resources and the support this lab gives. It takes the standards and creates a framework that allows teachers to support their students in their inquiry investigation.

Chemistry Teacher, CA

GREAT! I can use this for students to follow on their digital devices, and not have to waste paper.

Chemistry Teacher, CA

It's always great to incorporate technology in the classroom, I think this is powerful for any level of learner.

Biology Teacher, TX

carolina
science
ONLINE®

Time-Saving Digital Resources



Digital Teacher's Manual

A convenient, digital version of your teacher's manual that can be viewed on any device.



Downloadable Resources

Save a tree! These resources can be downloaded, printed, and emailed to your students.

Student Guide (PDF)

Fill-in Answer Sheet (PDF)

Editable Assessment Questions (DOCX)

Whiteboard Resources (PDF)

Glossary of Important Terms (PDF)

Visual Lab Procedure (PDF)

Real-World Connections (PDF)



Interactive Lessons and Simulations

Engage your students with interactive lessons that can be assigned and used on any device.

Strawberry DNA Extraction Prelab Activity

Strawberry DNA Procedure

DNA Structure and Organization

DNA to Protein Bingo



Websites

Expand learning with these websites.

Carolina Biotechnology Troubleshooting Guide

Objectives

Students' performance objectives are to

- use evidence to construct an explanation that all living things are made of cells, and therefore must contain DNA.
- understand cell wall and cell membrane composition.
- understand how cell barriers are broken and how to extract DNA from strawberry cells.
- develop a model to describe a causal account for the precipitation of DNA and the visualization of microscopic structures within the plant cell.
- use a model to describe how the structure of the plant cell wall, cell membrane, and nuclear membranes function together to compartmentalize cell function and to control what enters and leaves the cell.

Prerequisite Knowledge and Skills

- Students should be familiar with the basic structure and function of the organelles that comprise the eukaryotic cell, including the nucleus, nuclear membrane, cell membrane, and the cell wall in plant cells.
- Students should be familiar with the fluid mosaic model of the cell membrane, including the basic structure and function of the phospholipid bilayer.
- Students should be familiar with the packaging and basic structure of the DNA molecule.

Additional Student Resources

Real-World Connections

How does this kit relate to real-world applications and scientific careers? The Real-World Connections resource section, found as an appendix to this manual and on Carolina Science Online, provides you and your students with information to connect the knowledge and skills from this lab to the real world. On its pages you will find common uses for featured lab techniques, career connections, historical perspectives, and a scientist profile to help all students see themselves in science. You may wish to copy or print the document and distribute it as a reading assignment or additional resource.

Visual Lab Procedure

Because there are different types of learners, we include in the Student Guide a Visual Lab Procedure in addition to the textual version of the instructions. The Visual Lab Procedure contains images and an abbreviated version of the lab procedures and techniques.



Time Requirements

Teacher Preparation	40 minutes
Prelab	30 minutes
Investigation	60 minutes
Assessment	30 minutes



Safety

Use this kit only in accordance with established laboratory safety practices, including appropriate personal protective equipment (PPE) such as gloves, chemical splash goggles, and lab coats or aprons. Ensure that students understand and adhere to these practices.

Know and follow all federal, state, and local regulations as well as school district guidelines for the disposal of laboratory wastes. Students should not eat, drink, or chew gum in the lab and should wash their hands after entering and before exiting the lab.

Ethanol is a highly flammable liquid and vapor; keep it away from potential sources of ignition.

Download Safety Data Sheets (SDS) at carolina.com/sds or scan this code:




Materials

Included in the kit:

- ☐ 50-mL tubes with lids and bases, 17
- ☐ 15-mL tubes with lids, 33
- ☐ resealable plastic bags, 16
- ☐ wooden sticks, 16
- ☐ funnels, 16
- ☐ pack of cheesecloth
- ☐ transfer pipets, 16
- ☐ ethanol, 95% (100 mL)
- ☐ liquid detergent
- ☐ 10 g salt (NaCl)
- ☐ Digital Resource Instruction Card
- ☐ Teacher's Manual and Student Guide

Needed, but not supplied:

- ☐ scissors
- ☐ container (400-mL capacity or more) for mixing extraction buffer
- ☐ transfer vessels (5-mL capacity or more) for 95% ethanol, 16
- ☐ ripe, whole strawberries, 16 (fresh or frozen)
- ☐ 380 mL of water
- ☐ graduated cylinder or other device for measuring water
- ☐ freezer, refrigerator, or bucket of ice for chilling 95% ethanol
- ☐ spool of thread for demonstration



Using Digital Resources

- Preview the digital resources that come with the kit. These resources may be assigned to students to view or complete on their individual devices. They may also be projected for class instruction. Even the interactive lessons may be projected and completed as class activities.
- The Whiteboard Resources PDF includes photos and illustrations from the manual. Permission is granted to incorporate them into your laboratory investigations, class discussions, classroom presentations, and assessments.
- Tutorials supporting the full integration of the digital resources and the Carolina Science Online platform into your classroom lessons are available at <https://carolinascienceonline.com/#/help?tab=assignments>. Even if your students do not set up accounts on CSO, you may still project the digital resources for your class.

Preparation

One Day Before the Investigation

1. Make certain you have appropriate personal protective equipment for every student in your class.
2. a. Review the content of the Teacher's Manual and the Student Guide. Familiarize yourself with the instructions, materials, and assessments. Review the classroom management procedures and the timing of the materials setup steps.
b. Review the timing, content, and learning objectives of the discussion questions presented in the Laboratory Investigation Procedure. These questions have been developed to enhance students' understanding of the structure and function of plant cell organelles, and the physical and chemical process of extracting DNA from the nucleus of plant cells.
3. Photocopy or print the Student Guide for each student. Digital versions of Student Guide content are available online. If desired, also reproduce the Glossary of Important Terms. (Terms that appear in the Glossary are boldfaced in the Student Guide at first significant mention.)
4. Gather the materials that are needed but not supplied.
5. Chill the 95% ethanol by storing it in a freezer (preferred), refrigerator, or on ice until use.
6. If using frozen strawberries, allow the berries to thaw at room temperature before use. Do not heat the strawberries to hasten thawing, as this will hinder DNA extraction.
7. Prepare 400 mL of DNA extraction buffer. Use one of the 50-mL tubes to measure 20 mL of detergent. Pour the detergent into a container along with 380 mL of water and the entire contents (10 g) of the salt pack. Stir to mix.

8. Aliquot 10 mL of extraction buffer into 16 15-mL tubes (the tubes are calibrated for measuring).
9. Prepare 16 5-mL aliquots of 95% ethanol in transfer vessels. An extra 15-mL tube is included for measuring the 5-mL volumes.
10. Unroll cheesecloth from the spool and cut 16 pieces approximately 6" long. (The spool is about 8" wide, so each piece should measure about 6×8 ".)

The Day of the Investigation

1. Set up a workstation for each pair of students, and stock it with the following items. Alternatively, have students retrieve these items from a central materials station at the beginning of the lab.

10-mL aliquot of DNA extraction buffer in
15-mL tube

5-mL aliquot of 95% ethanol in transfer vessel
resealable plastic bag

strawberry

50-mL tube

15-mL tube

piece of cheesecloth

funnel

transfer pipet

wooden stick

Science Background

DNA is the “Code of Life”

Deoxyribonucleic acid (DNA) can be considered the hereditary “code of life” because it contains the information that determines an organism’s traits and is transmitted from one generation to the next. DNA can be compared to a recipe or a list of instructions about how to create and maintain a specific living thing. The DNA in an individual’s cells contains unique genetic instructions about how to build and sustain that individual.

DNA can be removed from organisms’ cells through a common and useful scientific procedure called DNA extraction. In order to understand this process, it is useful first to identify the basic structures that hold DNA molecules within living things.

Cells Contain DNA

DNA is located inside the cells of all species. However, different organisms are made up of different types of cells. Animal, plants, protists, and fungi are comprised of eukaryotic cells. This means that these cells have a true nucleus, a membrane-bound organelle within which the DNA is contained. The nucleus of eukaryotic cells is the “control center” that directs all cellular activities. Prokaryotic cells (bacteria and archaea) do not have nuclei. In these cells, DNA exists as a long loop coiled

loosely within the cytoplasm of the cell.

The nucleus of a eukaryotic cell is surrounded by a nuclear membrane (also called a nuclear envelope) and the entire cell is bound by a cell membrane (also called a plasma membrane). These barriers are both made up of two layers of fatty, oily compounds called lipids. The most abundant types of membrane lipids are phospholipids. These molecules have hydrophilic (“water-loving”) heads linked by a phosphate group to two hydrophobic (“water-fearing”) tails. The formation and stability of cell membranes is based on the orientation of phospholipid molecules in an aqueous (watery) environment. In such surroundings, phospholipids form a barrier of two rows with their hydrophobic tails facing each other (away from water) and their hydrophilic heads pointed outward (in contact with the aqueous environment). This two-layered structure is known as a phospholipid bilayer (Figure 1). Protein and carbohydrate molecules are also embedded within the phospholipid bilayer of cell membranes to transport particular molecules into and out of the cell and to conduct cellular messages.

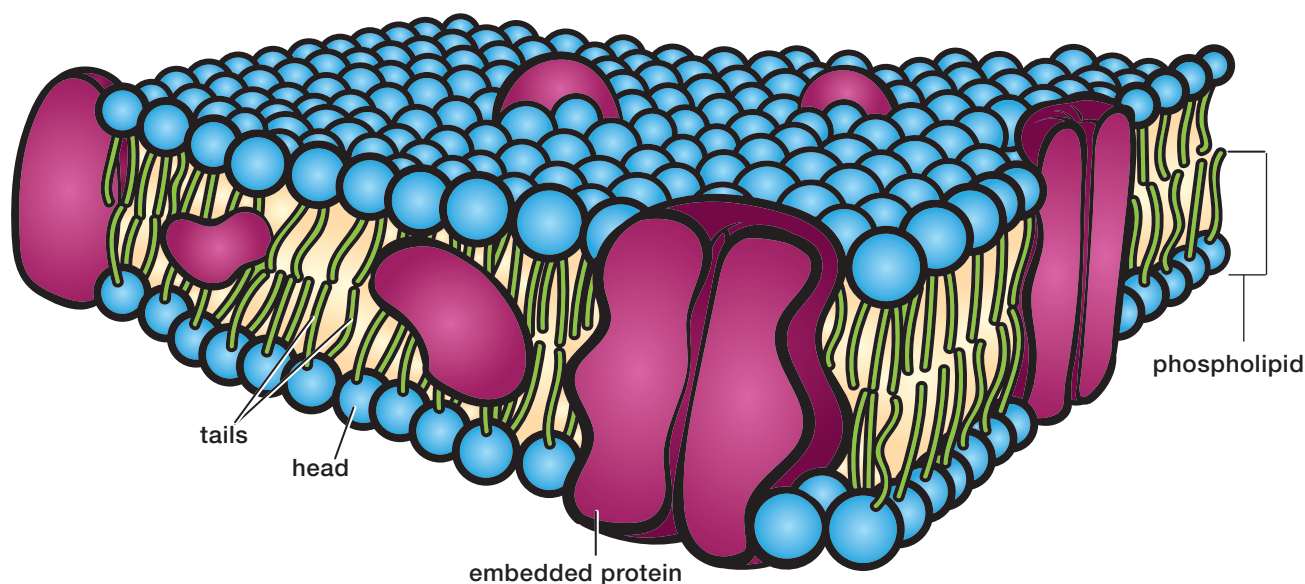


Figure 1. Phospholipid bilayer of cell membrane

All eukaryotic cells have a nuclear membrane that encircles the nucleus, as well as a cell membrane that encases the entire cell. However, plant cells (and some bacterial, fungal, and protist cells) have an additional barrier surrounding the cell membrane called a cell wall. Animal cells do not have cell walls. Plant cell walls are made of cellulose, a sturdy polysaccharide material comprised of glucose units. Cellulose gives plants their rigidity and provides a tough barrier that enables plant cells to hold a great deal of fluid without bursting.

Packaging and Structure of DNA

The DNA of eukaryotic cells is about 100,000 times as long as the cells themselves. However, it takes up only about 10% of the cells' volume. This is because DNA is highly convoluted (folded) and packaged as structures called chromosomes within cell nuclei. A chromosome is a bundle of tightly wound DNA coated with protein molecules. An organism's chromosomes bunch together within the nucleus like a ball of cotton, but during cell division (mitosis) they become individually distinct (human mitotic chromosomes are X-shaped) and can be observed with microscopes. DNA is not visible to the unaided eye unless it is amassed in large quantity by extraction from a considerable number of cells.

When chromosomal DNA is unfolded and the proteins coating it removed, the structure of DNA is exposed as a twisted ladder called a double helix. The sides of the ladder form the DNA backbone with alternating sugar and phosphate molecules linked by covalent bonds. The rungs of the ladder are comprised of pairs of nitrogenous bases [adenine (A) with thymine (T) and cytosine (C) with guanine (G)] joined by hydrogen bonds (Figure 2). Although the structure of DNA is well known and clearly defined, even the most powerful microscopes cannot visualize the DNA double helix of chromosomes.

All living things are dependent on DNA, and the structure of DNA is consistent among all species. However, the particular sequence of nitrogenous bases within DNA molecules differs between organisms to create explicit "blueprints" that specify individual living things. This sequence of base pairs is what makes an organism an oak tree instead of a blue jay, a male instead of a female, and so on.

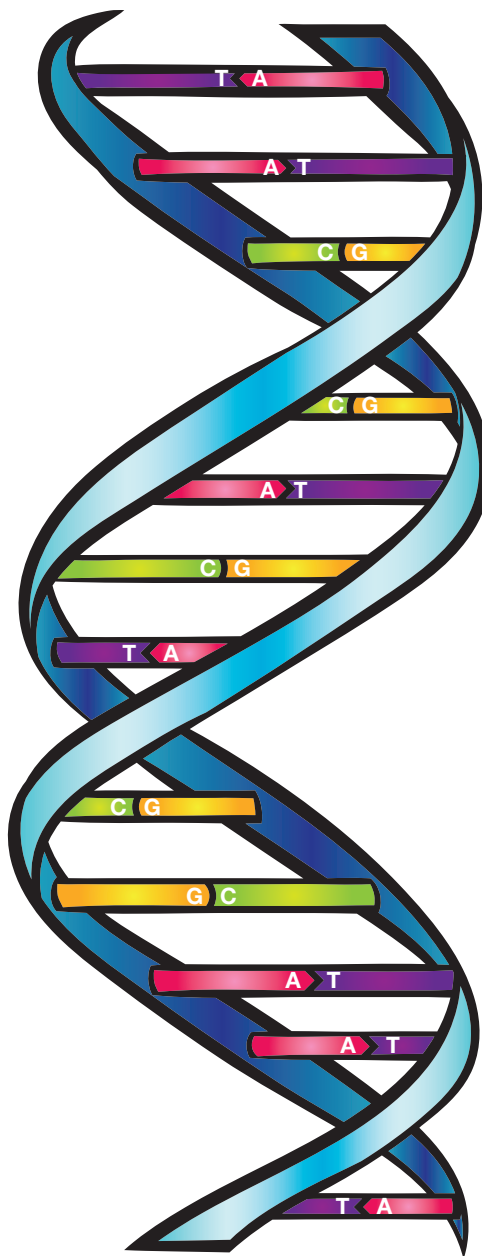


Figure 2. Double helix structure of DNA showing paired nitrogenous bases represented by letters G, C, A, and T

DNA Extraction from Plant Cells

The DNA of a plant cell is located in the cell's nucleus. The nucleus is surrounded by a nuclear membrane and the entire cell is encased in both a cell membrane and a cell wall. These barriers protect and separate the cell and its organelles from the surrounding environment. Therefore, in order to extract DNA from plant cells, the cell walls, cell membranes, and nuclear membranes must first be broken. The process of breaking open a cell is called cell lysis. Physical actions such as mashing, blending, or crushing the cells cause their cell walls to burst. The cell membranes and nuclear membranes may then be disrupted with a detergent-based extraction buffer. Just as a dishwashing detergent dissolves fats (lipids) to cleanse a frying pan, a detergent buffer dissolves the phospholipid bilayer of cell membranes. It separates the proteins from the phospholipids and forms water-soluble complexes with them. Once the cell wall and cell membranes are degraded, the cell contents flow out, creating a mixture of DNA, cell wall fragments, dissolved membranes, cellular proteins, and other material. This "soup" is called the lysate or cell extract.

DNA molecules are then isolated away from the cell debris in the lysate. For this purpose, the detergent-based extraction buffer also includes salt. The salt causes some of the cellular debris in the soup (such as proteins and carbohydrates) to precipitate out of solution while the DNA remains dissolved. This means that the cell debris become suspended particles that can be seen. The cell extract is then filtered through layers of cheesecloth. The cheesecloth traps the precipitated cell debris while the soluble DNA passes through. DNA is soluble in the aqueous cellular environment and in the presence of the extraction buffer, but is insoluble in alcohol (such as ethanol and isopropanol). Placing a layer of ethanol on top of the filtered lysate causes the DNA to precipitate out of the solution, forming a translucent cloud of fine, stringy fibers at the point where the alcohol and cell extract meet. Cold ethanol works best to precipitate DNA. DNA extracted from multiple cells becomes visible and can be wound onto a stick in a process known as "spooling" the DNA.

Strawberry cells are excellent sources of DNA for extraction in the classroom. They are multicellular and octoploid. This means they have eight copies of their seven chromosomes in each of their many cells. Therefore, just one berry will yield enough DNA to be easily seen and spooled. Strawberries are also a soft fruit, which makes them easy to mash. Mashing the berries breaks down the strawberry tissue, releases the individual cells, separates the seeds from the cells, and breaks the cell walls. In addition, ripe strawberries produce pectinase and cellulase—enzymes that contribute to the breakdown of cell walls.

Importance of DNA Extraction

DNA extraction is a fundamental procedure in scientific laboratories around the world. By extracting DNA, scientists can learn how DNA encodes the instructions for all life processes. DNA extraction is important to the study of heredity and to the treatment of many diseases through the creation of gene therapy DNA molecules. Extracted DNA can also be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war, and establish paternity or maternity. Scientists can genetically engineer changes in DNA to create robust, disease-resistant genetically modified plants and animals. DNA extraction is also necessary to sequence the order of base pairs of organisms (as in the Human Genome Project) and compare different species.

Module 3: Strawberry DNA Extraction **Teacher/Leader**

Target Audience: 7-12 Life Science, Biology, Ag Science

Overview:

In this lab, students will extract DNA from a strawberry using everyday materials and observe its physical appearance.

Objectives:

As a result of participating in this activity, students will:

- Know how to extract DNA from strawberries.
- Observe what DNA looks like to the naked eye.
- Learn that DNA is found in every living and once living thing.
- Understand that DNA is found in all the food we eat.

National Science Education Standards:

The following information was obtained from the *National Science Education Standards*. National Research Council, copyright 1996, National Academy Press.

Content Standard C: Life Science 5-8

- **Reproduction and Heredity.** Every organism requires a set of instructions for specifying its traits. Heredity is the passage of these instructions from one generation to another.

Content Standard C: Life Science 9-12

- **The Cell.** Cells store and use information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.
- **The Molecular Basis of Heredity.** In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular “letters”) and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome.

Science Content:

To show students a method to extract DNA from strawberries.

Science Process Skills:

- Observing
- Communicating
- Inferring

Life Skills:

- Communicating
- Learning
- Collaboration

Time:

50 minutes

Materials:

- Worksheets: Student pre-lab, DNA extraction lab, student response.
- 1 Zip-type, freezer bag (6"x 9")
- 1 Coffee filter, cone-shaped, #2 size
- 1 Plastic cup, 5 oz.
- 1 Plastic pipette
- 1 Strawberry
- 10 mLs DNA extraction buffer (soapy, salty water)
- 15 mLs ice cold ethanol in test tube

Lab Preparations:

The following solutions should be prepared by the instructor in advance of the lab.

DNA extraction buffer

Materials:

- 50 mLs of a clear hair shampoo with EDTA (Ex. Suave). Do not use one that contains a conditioner.
- 1 tsp of NaCl (table salt)
- 450 mLs water

In a one-quart jar or beaker, gently mix the materials so as not to create a lot of bubbles. This will make enough extraction buffer for 50 groups of two students. Provide pipettes for students to draw 10 mLs of the extraction buffer from the jar to add to their plastic bags with mashed strawberries in step three of part one.

Ethanol preparation

Materials:

- 750 mLs of 93% Ethanol or 95% Ethanol (Store in a refrigerator to keep cold.)
- Graduated cylinder or 25 mL pipette
- Ice
- Container for the ice
- Test tubes with corks or caps

Fill 50 test tubes with 15 mLs of ethanol and cap. 750 mLs of ethanol will be enough for 50 groups of two students. You may store the filled test tubes in a non-food refrigerator until class time. Put capped test tubes in ice to keep the ethanol as cold as possible during the lab. The cold ethanol helps the DNA to precipitate out of solution.

Organization tips:

A good way to help organize lab materials for students is to sort the necessary items into plastic boxes labeled with their group number. Use boxes about the size of a standard shoebox. (Regular shoeboxes work as well.) This saves time during class and you know that the students will have the necessary items. Replace the consumed items after each class.

Example of contents of student boxes for this lab:

- 1- Pipette
- 1- Cone-shaped coffee filter
- 1- 5 oz. Plastic cup
- 1- Plastic freezer bag

Lesson Guide:

Anticipatory Set:

1. Handout pre-lab worksheet, "Strawberries & DNA". Part I: Ask students to list what they know about strawberries and DNA.
2. Discuss their answers to Part I.
3. Part II: Have the students list what their individual questions are about strawberries and DNA.
4. Then, with their partners, have students write one or two questions about strawberries or DNA or both and write on a sheet of colored paper to share with the entire group. Turn questions in to the teacher. These can be put on a large sheet of paper or on the chalkboard, to be answered when appropriate during class or at the end of class.

Lab Introduction:

5. Tell students that today they will have the chance to see what actual DNA looks like by extracting DNA from a strawberry. (Encourage students to write down any new questions as they do the lab, and share them with the group.)
6. Hand out to the students the "Strawberry DNA Extraction" and "Student Response" worksheets.
7. Have the students read the introduction section of the "Strawberry DNA Extraction" worksheet. Be sure to show the plant cell and review its parts.
8. How do we isolate DNA from the cell? (What is the method? It is called DNA Extraction.) To understand how DNA extraction is done, you will extract DNA from strawberries today.

Lab:

1. See lab handout "Strawberry DNA Extraction"

Questions:

1. Have students complete the "Student Response" worksheet.
2. Answer any questions from the list made at the beginning of class.

Closure:

1. Ask students what they learned about DNA and strawberries.
2. Ask students where DNA is found.
3. Ask them what the four main steps were of the DNA extraction.
 - Crushed the cells to release the DNA.
 - Used the buffer to separate the DNA from the other cell components.
 - Filtered out the large particles.
 - Precipitated out the DNA using ethanol.
4. Ask the students what the DNA looked like.

For more information about DNA, check out the website listed below:

<http://www.dnaftb.org/dnaftb/29/concept/index.html>

Acknowledgement:

The Strawberry DNA Extraction activity, lab protocol and student worksheets were modified and used with permission from Julie Townsend, Parkview Middle School, Ankeny, IA.

Strawberries & DNA



I. List what you know.

What do you know about strawberries?	What do you know about DNA?

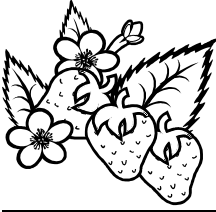
II. List what you want to know.

What do you **want to know** about DNA?
strawberries?

What do you **want to know** about

III. Share with your partner and create one or two questions about strawberries, DNA, or both.

IV. Write the question(s) on the colored paper strip to share with the entire group.



Strawberry DNA Extraction

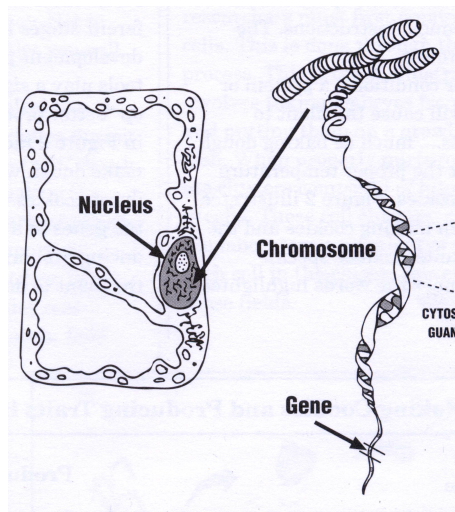
Introduction:

Have you ever wondered what DNA looks like? You are going to break apart the cell membrane of a strawberry and separate the DNA from the nucleus. Strawberries are a good source of DNA because they have 8 copies of each type of chromosome. This large number of chromosomes will filter out of your solution and you will get to actually see DNA.

Review:

Where in the cell is the DNA found?

Take a look at the sketch of the plant cell below. The chromosomes (which are made of DNA) are in the nucleus. This is the place where most of the DNA is located. (There are also small amounts of DNA in the chloroplast and mitochondria.)



What do you think the DNA will look like when you extract it from the plant cell? (Write a brief description.)

DNA Extraction from Strawberries

Materials:

Lab box contents:

- 1 Zip-type, freezer bag (6"x 9")
- 1 Coffee filter, cone-shaped, #2 size
- 1 Plastic cup, 5 oz.
- 1 Plastic pipette

Available from your teacher:

- 1 Strawberry
- 10 mLs DNA extraction buffer (soapy, salty water)
- 15 mLs ice cold ethanol in test tube

Procedure:

Working together in groups of two, read through the entire procedure and follow the steps in Parts I, II and III.

Part I- Preparing the DNA extract

1. Have one partner get the lab box while the other partner gets the strawberry and removes the stem and leaves.
2. Place one strawberry in a zipper bag.
3. Mash up the strawberry for 2 minutes.
4. Add 10 mLs of the **extraction buffer** to the bag.
5. Mash again for 1 minute. While one partner is mashing the other partner must do step 6.
6. Drape the coffee filter in the cup. Make sure the top part of the filter is folded over the top of your cup and that the bottom of the filter does not touch the bottom of the cup.
7. Pour the mashed strawberries with the extraction buffer into the filter and let drip into the bottom of the cup. This takes about 10 minutes.

As you wait for your solution to filter, complete the following questions:

Part I: Questions

- a. What was the purpose of mashing up the strawberry?
- b. What does the extraction buffer do? (Hint: Extraction buffer contains soap. What does soap do when you wash your hands?)
- c. What does the filter do?

Part II: Pipetting the strawberry extract into the alcohol

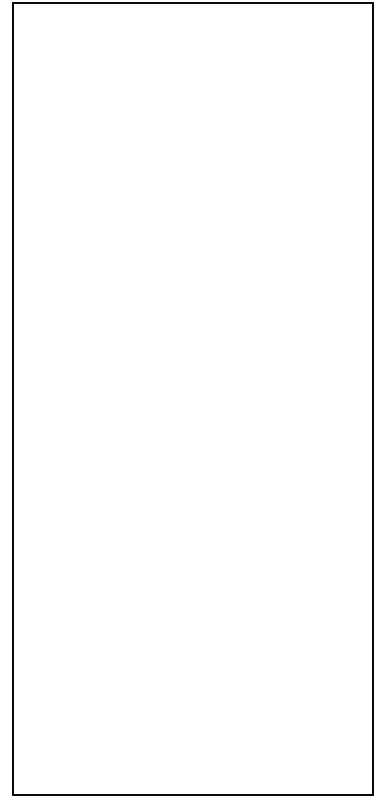
8. One partner gets a test tube that contains **ice cold ethanol**.
9. Using a pipette, remove some of the strawberry extract from the cup. Carefully pipette the strawberry extract into the alcohol in the test tube and watch the solution precipitate (separate).
DO NOT SHAKE THE TUBE!!! Very gently swirl the tube once or twice. Then let the tube remain undisturbed.

Part III: Observations

10. Watch where the alcohol and extract layers come in contact with each other. Keep the tube at eye level so you can see what is happening.

What do you see appearing? (Sketch what you see in the box and note any other observations.)

Notes:



Questions Parts II and III:

- a. What happened when you added the filtrate to the alcohol?
- b. What did the DNA look like?

Name: _____
Student Number: _____
Date: _____
Lab Partner's Name: _____

Strawberry DNA Student Response



1. What does DNA look like?

2. A person cannot see a single cotton thread four classrooms away. But if you wound thousands of threads together into a rope, it would be visible at the same distance. How is this statement an analogy to our DNA extraction?

3. Is DNA found in all living or once living cells?

4. Since the strawberries were once living, and we extracted DNA from them, what does this mean about the foods you eat?

5. Look at the plant cell pictured on page one of the lab handout. Remember that genes are found on chromosomes, and genes control traits. Give at least two examples of traits that are expressed in the strawberry.

6. Since we are studying about Bt and non-Bt corn in this unit, name at least two traits a scientist would be interested in having in corn.

Answer Key for pages 6-9.

Page 6:

Part I: Questions

- a. What was the purpose of mashing up the strawberry?

To break down the cell wall, cellular and nuclear membranes.

- b. What does the extraction buffer do? (Hint: Extraction buffer contains soap. What does soap do when you wash your hands?)

The extraction buffer helps to release the DNA from the surrounding cell components of the crushed strawberry.

- c. What does the filter do?

The filter removes the larger particles from the solution, such as seeds, pith, etc., allowing only the smaller cell components such as the DNA, proteins, etc. to filter through.

Page 7:

Questions Parts II and III:

- c. What happened when you added the filtrate to the alcohol?

The DNA precipitated out of the solution.

- d. What did the DNA look like?

The DNA looked like white, thin fibers wadded up together forming a clump.

Pages 8-9:

1. What does DNA look like?

The DNA will look like a white, cloudy or fine stringy substance.

2. A person cannot see a single cotton thread four classrooms away. But if you wound thousands of threads together into a rope, it would be visible at the same distance. How is this statement an analogy to our DNA extraction?

DNA is not visible as a single strand to the naked eye, but when thousands of threads of DNA are present, you will be able to see the large groups of threads of DNA.

3. Is DNA found in all living or once living cells?

Yes, DNA is present in all living and once living cells. Scientists can use DNA from mummies or seeds that are thousands of years old and identify the genetics of those plants and animals using their DNA.

4. Since the strawberries were once living, and we extracted DNA from them, what does this mean about the foods you eat?

All the foods we eat that come from plants and animals that contain DNA. DNA has been consumed on a daily basis since the beginning of time and is completely safe to eat.

5. Look at the plant cell pictured on page one of the lab handout. Remember that genes are found on chromosomes, and genes control traits. Give at least two examples of traits that are expressed in the strawberry.

Examples: Color, size and sweetness.

6. Since we are studying about Bt and non-Bt corn in this unit, name at least two traits a scientist would be interested in having in corn.

Examples: Yield, drought resistance, insect resistance, disease resistance, stalk strength, root strength, early growth, stays green, days until maturity, and height.

Strawberry DNA Extraction

1. Obtain one fresh or one frozen and thawed strawberry. If you are using a fresh strawberry, remove the green sepals (tops) from the berry.
2. Place the strawberry in a resealable plastic bag.
3. Close the bag slowly, pushing all of the air out of the bag as you seal it.
4. Being careful not to break the bag, thoroughly mash the strawberry with your hands for two minutes.
5. Pour the 10-mL aliquot of extraction buffer into the bag with the mashed strawberry. Reseal the bag.
6. Mash the strawberry for one additional minute.
7. Place a funnel into a 50-mL centrifuge tube. Fold the cheesecloth in half along the longer side and place it in the funnel to create a filter. The cheesecloth will overlap the edge of the funnel.
8. Pour the strawberry mixture into the funnel, filtering the contents through the cheesecloth and into the 50-mL centrifuge tube.
9. Carefully pour 2 mL of the filtered contents from the 50-mL tube into a clean 15-mL tube. Use the lines on the side of the 15-mL tube to help measure the amount added.
10. Hold the 15-mL tube at an angle. Using a transfer pipet, carefully add 5 mL of cold 95% ethanol by running it down the inside of the tube. Add the 95% ethanol until the total volume is 7 mL (use the lines on the side of the tube to help you measure). You should have two distinct layers.
Caution: Do not mix the strawberry extract and the ethanol!
11. Watch closely as translucent strands of DNA begin to clump together where the ethanol layer meets the strawberry extract layer. Tiny bubbles in the ethanol layer will appear where the DNA precipitates.
12. Slowly and carefully rotate the wooden stick in the ethanol directly above the extract layer to wind (or "spool") the DNA. Remove the wooden stick from the tube and observe the DNA (see Figure 1: DNA Extraction).

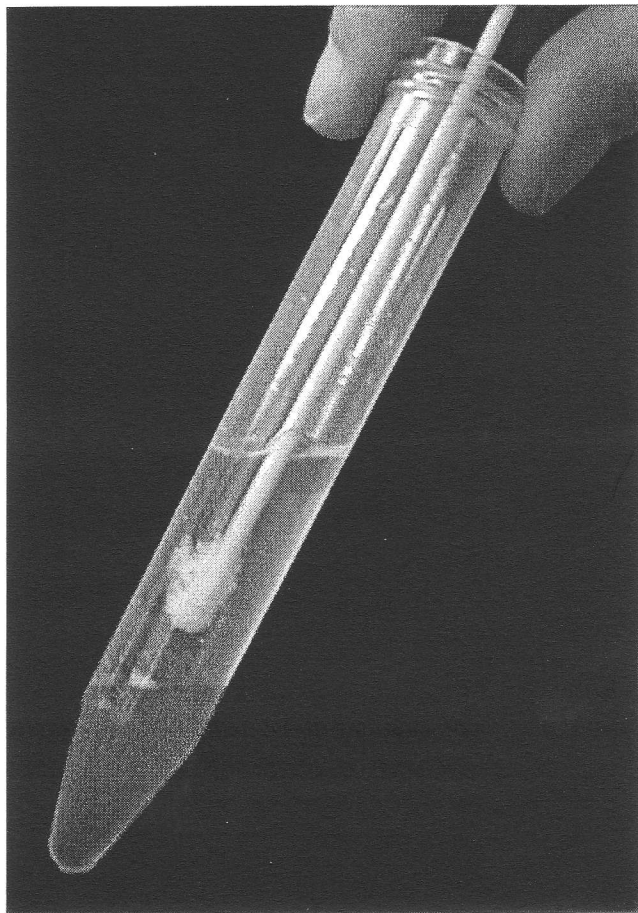
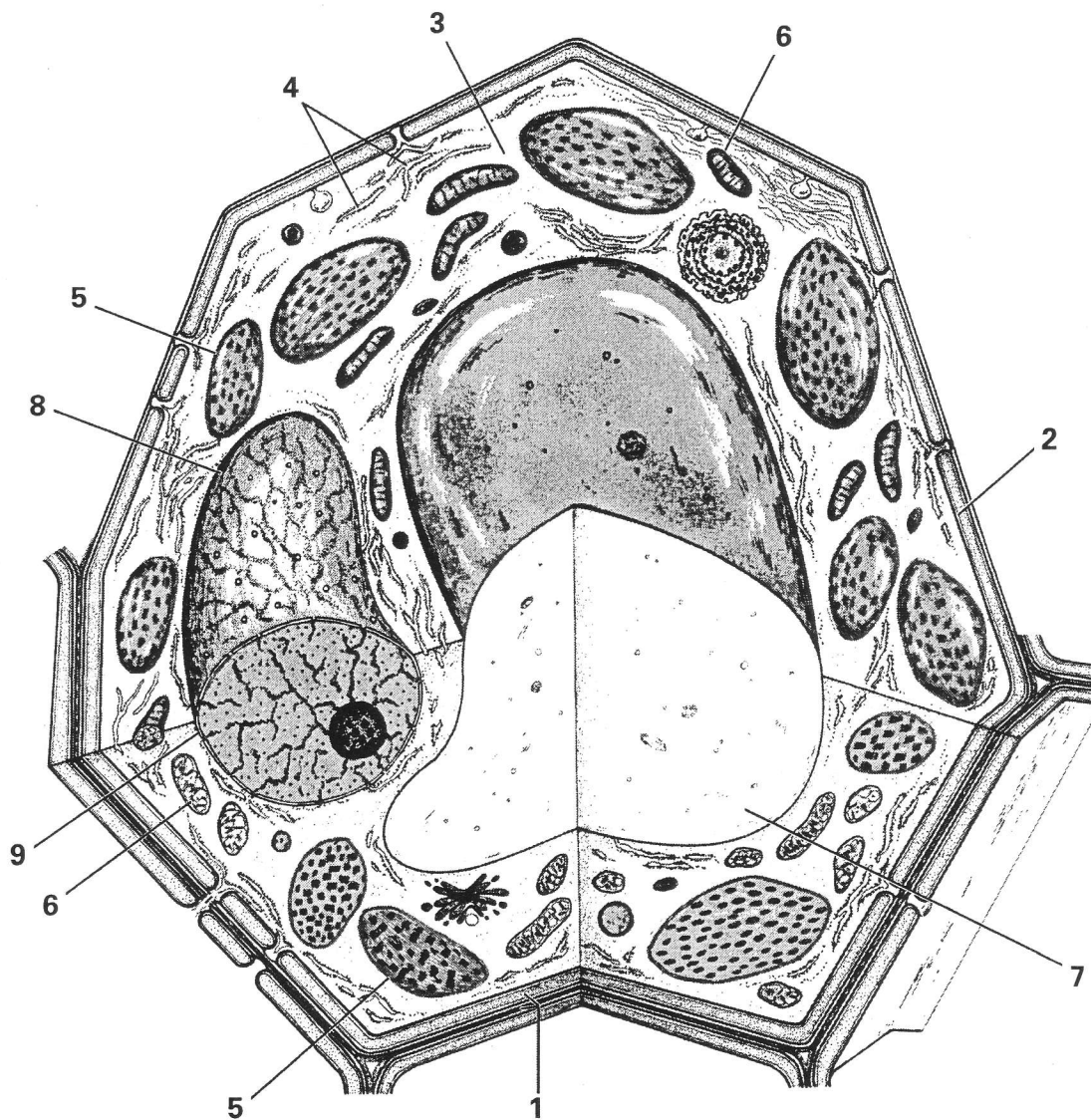


Figure 1. DNA Extraction

Strawberry DNA Extraction Review

1. In your own words, describe the structure and function of DNA.
2. What does mashing the strawberries and treating them with detergent do to their cells?
3. Name a liquid that DNA is not soluble in. What does it mean to be insoluble?
4. If you had extracted DNA from animal cells instead of plant cells, what cell barrier would have been different? What do plant and animal cells have that bacterial cells do not?
5. Do you think that DNA from animal cells would look the same as DNA from plant cells?
6. Give one example of something a scientist can do with extracted DNA.

Plant Cell Diagram



1. _____

2. _____

3. _____

4. _____

5. _____

6. _____

7. _____

8. _____

9. _____