NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Snow White Apples?

RNAi and Genetically Modified Foods

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Part I – Making Arctic Apples

"Hi Dad, I'm home!" Maria slammed the door. "Can you bring me to the field early? I want to make sure I have time to warm up before the soccer game." Her brother Juan smiled to himself from a chair in the living room; Maria had always put soccer before everything else.

Dad stuck his head out from his home office. "Sure, but I need you to finish your afternoon check list first—unpack your lunch, get a snack, check the chore chart….and get it done before we go."

Maria sighed. "I don't understand why I have to do all this stuff. It isn't like I don't have other things to do, like my science project and math homework."

Dad chuckled under his breath. "I guess it's hard being a teenager. How about I come to the kitchen with you, and we can get a snack and unpack your lunchbox together? We can talk about your science project while we do it."

Juan jumped out of the chair and startled his sister. "Hi Maria! My class tomorrow is canceled, so I decided to come home for the night. Maybe I can drive you to practice…after you finish Dad's to-do list."

"Hey, it's great to see you! And it would be great to hang out with you!" Maria commented as she walked into the kitchen with her lunchbox in her hand and backpack hanging open. She gave her lunch box to her father and dug out her science notebook. "Maybe you can help me with my biology homework. Our project due at the end of the semester needs to include some aspect of molecular biology, and there are bonus points if it includes something about plants."

Dad opened her lunchbox. "Maria, why didn't you eat your apple slices? I cut them because I know it's your favorite fruit, but you can't eat whole ones because of your braces. Don't you like apples anymore?"

"Dad, did you *see* those apples? They're all brown and gross! Would *you* want to eat them?"

"Hmm. They are pretty brown. And it looks like being smashed with your books didn't help," Juan commented. "How about starting over. I'll cut some fresh apple slices. Maria, have you talked about the central dogma of biology? That's the basis of molecular biology. We just talked about it in my introductory biology class. Why don't you watch these videos while you eat your apple slices?"

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Watch the video, Transcription and Translation (HHMI Biointeractive Compiled Video): <https://youtu.be/0VA275plaQE>

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"So, Maria, how were your apple slices?" Dad asked as he unloaded the dishwasher. "Did you learn something from the video?"

"They were pretty good, though they would have been better with peanut butter or caramel," Maria sighed.

"Ha! So you *do* like brown apples—just brown with condiments!" Juan interjected.

Maria laughed and continued. "The video was interesting. DNA is the master recipe book, and RNA is like the directions for making proteins that do the jobs in a cell."

"That's right," Juan chuckled, "though how it all is regulated makes it a bit more complicated."

"Hey, I just had an idea!" Maria exclaimed. "Maybe I could do my project on the protein that makes the apples turn brown! Those little baggies of apples at the fast food restaurants never seem to turn brown, so there must be a way to stop it. And apples come from plants, so it covers the plant part of the assignment." Maria grabbed her smart phone and said, "Siri, why do apples turn brown?" Her phone promptly replied:

> When an apple is cut (or bruised), oxygen is introduced into the injured plant tissue. When oxygen is present in cells, polyphenol oxidase (PPO) enzymes in the chloroplasts rapidly oxidize phenolic compounds naturally present in the apple tissues.
>
> —*L. McLandsborough, "Why do apple slices turn brown after being cut?"* Scientific American *July 30, 2007.* <*http://www.scientificamerican.com/article/experts-why-cut-apples-turn-brown/*>.

"Hey, that '–ase' at the end of *oxidase* means it's an enzyme, and enzymes are proteins! And since proteins are made from mRNA, maybe I can make a connection to the central dogma and get the extra credit!" Maria was more than a little excited.

"Well, it sounds like you have an interesting idea, Maria. But we need to leave now if we're going to make it to practice on time," Juan said.

"And don't forget to put your dishes in the dishwasher," called Dad from the hallway.

Maria continued the conversation as she climbed into the car. "I know that when we eat Golden Delicious apples, they turn brown almost instantly, but the Granny Smiths stay white longer. Maybe they have different amounts of polyphenol oxidase in them? Ooh, I wonder if there are any apples that don't turn brown at all?" Juan smiled at her excitement. Maria picked up her smart phone again, and said, " 'Siri: apples that don't turn brown.' Hey, I got some hits! And the first one is a genetically modified apple that doesn't turn brown at all. Check out this picture:"

Figure 1. Left: conventional apple. Right: Arctic variety.
Credit Okanagan Specialty Fruits, with permission.

"Well, that would solve your lunchbox problem. Tell me more about how they're made," Juan replied.

"They genetically modify the apple, and use RNA interference to silence the polyphenol oxidase. Whoa. Those are some pretty technical topics. Maybe I'm okay with apples that turn brown," Maria said.

"You should understand it before you judge it. And it fits your molecular biology requirement," said Juan. "We're learning about these processes in my biology class right now. Let me do some research while you're at practice, and I'll share what I learn with you later."

Pre-Class Assignment for Part I

Name: _____

Bring your completed worksheets to class.

1. Propose two different ways that the amount of protein could be controlled in the cell.

2. Watch the following video on RNA interference and read the appropriate pages in your textbook, and answer the questions below. <https://youtu.be/cK-OGB1_ELE>
   a. Where in the cell does each of the following processes occur?
      i. Transcription: _____
      ii. Translation: _____
      iii. RNA interference (RNAi): _____
   b. Define, then compare and contrast siRNA and miRNA.

   c. Define the following terms or abbreviations, and explain their role in RNA interference:

      DICER:

      Argonaute:

      RISC:

3. Make a flow chart of the steps of RNAi; be sure to include DICER, Argonaute, and RISC from the preceding question.

4. List one advantage and one disadvantage of regulating protein expression via RNAi vs. transcriptional regulation.

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Watch the following video before continuing to answer the questions below: *How to Make a Genetically Modified Plant,* <https://youtu.be/JtkhHIG3nx4>.

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5. What are the four main components needed to make a transgenic plant? Summarize the function of each component.

6. Compare and contrast (two each) the two methods for getting DNA into a cell.

7. The DNA that is used in the gene gun method is usually cut out of a plasmid that has been grown in *E. coli* cells. Sketch a plasmid map for this plasmid.

8. Why are two different selection genes required in the Ti plasmid?

9. Why are the virulence genes included in the Ti plasmid when used for engineering plants?

10. Which Ti plasmid DNA sequences would you expect to find in a transgenic plant? Explain.
    a. Virulence genes:

    b. 35S promoter:

    c. NOS termination sequences:

    d. Left and right borders:

    e. Selection gene with *Agrobacterium* promoter:

    f. Selection gene with a plant promoter:

    g. Opine synthesis genes:

    h. Origin of replication (ORI):

In-Class Activity for Part I

1. Compare the plasmids you designed for Question 7 on the Homework worksheets. Which of the components from Question 10 should be present in the gene gun DNA source plasmid? Sketch your consensus plasmid below.

2. Describe the required components for RNAi. Which of these components are present in cells? Which would you need to add to the cells?

3. What is the advantage of introducing the RNAi required components via genetic modification, as opposed to another method for delivering double stranded RNA?

4. You have been hired by Okanagan Specialty Fruits to develop Arctic Apples, which are genetically modified to silence polyphenol oxidase via RNAi. Brainstorm with your group the process you will follow to make and test Arctic Apples.

5. Apples contain at least eleven polyphenol oxidase genes, organized into four gene families: PPO2, GPO3, APO5 and pSR7; the mRNAs from these genes range in size from 1350 bp to 2000 bp. The silencing plasmid contains a 200 bp segment from each of the four consensus mRNA sequences.
   a. Why is it possible to only use a portion of the PPO genes in the silencing plasmid?

   b. What are the advantages of using a smaller section of the PPO genes?

6. Below is a map for the plasmid used to make Arctic Apples. Circle the region that is transferred into the plant and the key components of each section. Complete the table summarizing the function of each segment. (*Hint: P* before a name indicates a promoter.)

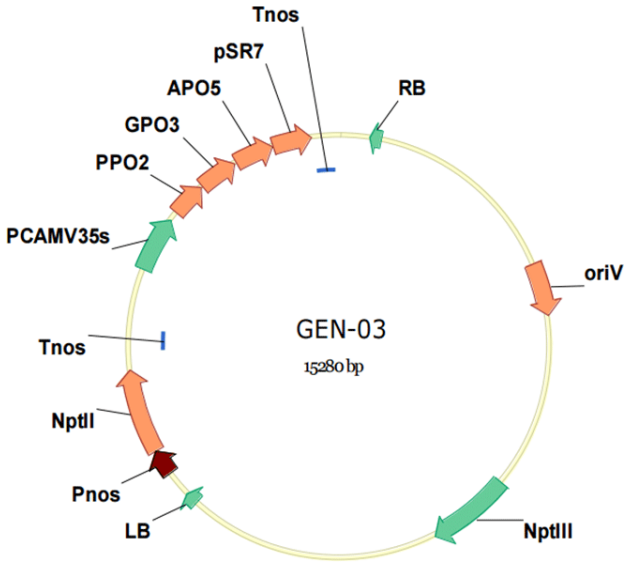

Figure 2. Map of the GEN-03 vector.

Genetic element	*Function*
LB	
P_{nos}	
nptII	Neomycin phosphotransferase type II; gives resistance to kanamycin
T_{nos}	
$P_{CaMV35S}$	
PPO2, GPO3, APO5, pSR7	200 bp sequences from the four different PPO gene family members
RB	

7. Wrap-up homework: write an email to Maria, summarizing how RNAi and genetic modification are used to silence PPO expression in Arctic Apples. Keep in mind that Maria is a high school student, so you will need to define and explain any terminology you learned during this case study.

Part II – Arctic Apple Concerns

Juan called his sister the next day. "Hey, Maria, what did you think of the information I sent you?"

"It was pretty cool! RNAi and genetically modifying plants isn't as scary as I thought! But isn't there a reason that plants make polyphenol oxidase? Is engineering the plant to not make it going to affect how it grows, or how it protects itself from disease? Would eating an apple that has silenced polyphenol oxidase affect me somehow, like silencing something in me? And what about the antibiotic resistance gene they use to select which plants have the transgenic modification—would that make problems with antibiotic resistance even worse?"

"Wow, that's a lot of questions, Maria. Why don't I ask some of my friends to help me research some answers for you?"

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Pre-Class Assignment for Part II

You will be assigned one of three roles to answer Maria's question. In your assigned role, you will read a paper as homework, answer questions about the paper, and write a note to Maria about what you learned. In class, you will exchange information with individuals who learned about the other topics, and discuss whether genetically modified foods should be freely available, labeled, or banned.

Microbiologists

- European Federation of Biotechnology, Task Group on the Public Perception of Biotechnology. 2001. Antibiotic resistance markers in genetically modified (GM) crops. *Briefing Paper #10.* <http://sciencecases.lib.buffalo.edu/cs/admin/Supplemental/UploadFolder/snow_white_flip_sup.pdf>.

Plant Biologists

- Carter, N. 2012. Petition for determination of non-regulated status: Arctic™ Apple (*Malus x domestica*) Events GD743 and GS784. Available at <https://www.aphis.usda.gov/brs/aphisdocs/10_16101p.pdf>. (Read pages 61–80 only.) (Also, for help with understanding the Chi-square test, see the following online tutorial: Stat Trek. *n.d.* Chi-square test for independence. <http://stattrek.com/chi-square-test/independence.aspx?Tutorial=AP>.)

Biosafety Specialists

- Roberts, A.F., Y. Devos, G.N.Y. Lemgo, and X. Zhou. 2015. Biosafety research for non-target organism risk assessment of RNAi-based GE plants. *Frontiers in Plant Science* 6: 958.

Microbiologist Handout

Read: Antibiotic resistance markers in genetically modified (GM) crops:
<http://sciencecases.lib.buffalo.edu/cs/admin/Supplemental/UploadFolder/snow_white_flip_sup.pdf>.

Make a list of confusing terms or concepts from your reading assignment, and define them below. You must define at least three terms:

_____ : _____

_____ : _____

_____ : _____

Questions

1. Why are antibiotic resistance genes used in making transgenic plants?

2. What are the concerns about the use of antibiotic resistance markers in making transgenic plants?

3. What steps would need to happen for antibiotic resistance genes in a plant to be transferred to a bacteria that infects humans?

4. How does antimicrobial resistance develop or transfer between bacteria?

5. Write a one paragraph summary of what you learned for Maria, explaining why she should or should not be concerned about the antibiotic resistance genes used in making transgenic plants.

Biosafety Specialist Handout

Read: Biosafety research for non-target organism risk assessment of RNAi-based GE plants" from *Frontiers in Plant Science* (2015) 6: 958.

Make a list of confusing terms or concepts from your reading assignment, and define them below. You must define at least three terms:

_____ : _____

_____ : _____

_____ : _____

Questions

1. Brainstorm all the possible non-target organisms that could be exposed to dsRNA from an Arctic Apple.

2. What steps would need to occur in order for the consumption of an Arctic Apple to lead to non-target gene expression in a human?

3. One of the concerns about using RNAi is the potential for off-target effects in humans, so we will use a Basic Local Alignment Sequence Search Tool (BLAST) to check.
   a. Go to https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch
   b. Every sequence in the National Library of Medicine has a unique identifier. Choose one of the following PPO sequences to analyze by entering the accession number in the first box (circle the one you select):

      JQ388482.1 JQ388479.1 L29450.1

   c. Select "Reference RNA sequences» in the Database box, and "Homo sapiens" in the Organism box. Click "Optimize for Somewhat similar sequences," then click submit.
   d. Comment on any hits you obtain. What kinds of functions do the protein(s) have? Are there any stretches of >21 contiguous exact matches? Any gaps?

   e. The PPO silencing constructs were made with just the first 200 bases of the coding region. Are your hits in this region?

   f. Is there a risk to human health via gene silencing from eating an Arctic Apple? Why or why not?

4. Write a one paragraph summary of what you learned for Maria, explaining why she should or should not be concerned about possible off-target effects.

Plant Biologist Handout

Read: Petition for determination of non-regulated status: Arctic™ Apple (*Malus x domestica*) Events GD743 and GS784. Available at <https://www.aphis.usda.gov/brs/aphisdocs/10_16101p.pdf>. (Read pages 61–80 only.)

Make a list of confusing terms or concepts from your reading assignment, and define them below. You must define at least three terms:

_____ : _____

_____ : _____

_____ : _____

Questions

1. What are the growth variables that were considered in the field trial?

2. Make a list of the pathogens that were monitored and classify them as insect, bacterial, or fungal. Did PPO silencing affect the tree's ability to fight off infection? How confident are you in that answer?

3. List two advantages and two disadvantages of using field trials to evaluate the effect of silencing PPO on plant growth and disease susceptibility.

4. Design a lab experiment to test the effect of PPO silencing upon an apple plant's ability to resist a particular pathogen (choose your favorite from Question #2).

5. Write a one paragraph summary of what you learned for Maria, explaining why she should or should not be concerned about the effect of silencing PPO on plant growth and ability to resist disease.

In-Class Activity for Part II

For the first half of class, you will meet with others of the same specialty group to go over your answers and to prepare a short (three minute) presentation on the main points of what you learned. Your talking points should outline:

a. What is the concern you explored?
b. What are the claims about that concern?
c. How has that claim been explored (experimentally or logically)?
d. How strong is the evidence for or against the claim?
e. In your opinion, is the concern a reason not to allow Arctic Apples on the market? Why or why not?

During the second half of the class period, you will meet with students from the other specialist groups to exchange what you learned and complete the grid below.

Topic	*What is the concern?*	*What would need to happen for it to be a problem?*	*Should you (or Maria) be concerned? Why or why not?*
Antibiotic resistance			
RNAi of human genes			
Problems with the apple tree's ability to fight off disease			

Part III — Labelling GM Foods

Pre-Class Assignment for Part III

To prepare for the in-class activity, please read <http://agbioforum.org/v1n1/v1n1a06-caswell.htm> and find an article of your own choosing. For the article of your own choosing, complete the worksheet below:

Article title: _____

Author: _____

Citation: _____

Find out more about the source, either by reading the "about us" or a brief biography of the author. Write one paragraph, summarizing why you think this is a valid source to consider for this discussion. Include the strengths, limitations, and potential biases for the source:

Write a one paragraph summary of the main points of this article:

In-Class Activity for Part III

The goal of this deliberative discussion is to get as many different ideas on the table, then discuss the consequences of each action, and make some decisions on which action or actions are best. We may not reach a consensus at the end of this discussion, and that is ok.

The format of the discussion will be as follows:

1. Each specialty area will share what they learned about a concern about RNAi transgenic foods for 2–3 minutes.
2. We will read over each of the options out loud and discuss factors relating to each option for approximately 10 minutes each. While your specialty area learning may inform your position, you are presenting your own opinions, not the opinions of that specialty area.
3. Within your group, assign the roles of time keeper (who enforces the time limits), manager (who keeps the conversation on track) and three note takers (one for each possible strategy).
4. We will re-convene as a class and summarize our discussion, and vote on the three possible outcomes.

In your discussion group, it is important that your contributions are backed by evidence, including evaluating sources; weighing tradeoffs and tensions; considering others' viewpoints and differences of opinions from different groups; and integrating all the information before coming to a decision. You will evaluate (and be evaluated by your peers) on your ability to do this.

Option 1–Labeling GM Foods

Labeling genetically modified (GM) foods in the United States has always been a hot topic. Americans are accustomed to being informed on what type of chemicals are in their foods, which could include information such as calories, sugars, and proteins, to chemicals such as phosphoric acid, sulfuric acid and ethylene. But should the American public be informed of the presence of GMs? Other major countries around the world, including countries in the European Union, require that GM foods be labeled. In the United States the Food and Drug Administration in a 1992 policy decision concluded that foods made with genetically modified organisms are not materially different from other products made without GMs. Is this sufficient evidence to conclude that GM foods should not be labeled? Even if we did label GM foods would this labeling be accurate, seeing that there have been many cases where genetic modifications are found in supposedly non-GM foods due to spread of genes through wind pollination?

Discussion Questions

1. Would labeling GM foods change the products you purchase? Why or why not?

2. On a larger scale, who would be affected by labeling GM foods? Would this affect them in a positive or negative way and why?

3. What would be the challenges with labeling GM foods?

Option 2–Not Labeling GM foods

A second option would be to simply remove all existing regulatory efforts with regards to the labeling of GM foods. Currently, there are proposals across all levels of government in America to require labeling of GMOs but the law in place is that food labeling must occur when "there is a substantial difference in the nutritional or safety characteristics of a new food." Other countries like England do require labeling of GM foods, which requires definition of what must be labeled and enforcement to ensure that all food is properly labeled. Citizens of England pay far more for their food

than we do in the United States, partially to pay for the labeling. The amount of money and time that would be saved by individuals in the government and in the food industry would be substantial if there was not a labeling requirement for GMOs. However, not requiring labeling of GM foods means citizens have less information to make decisions about the food that they are purchasing. Companies could voluntarily label their products, but there would be no mandate in place for this approach.

Discussion Questions

1. What are your initial impressions of not labeling foods about genetic modifications?

2. Do you think food companies would take advantage of voluntary labeling if there was no mandate?

3. Do you think it would be acceptable to drop all of the regulatory efforts for labeling GM foods in America? What would we be sacrificing? What would we be gaining? Is it worth a tradeoff?

Option 3–Banning GM Foods

Some people believe that genetically modified foods should not exist at all. Some people say we shouldn't play God with our food. Others are concerned about the effect of genetic modifications on the larger environment, as genetic modifications, especially herbicide resistance genes, change how farmers use herbicides, leading to changes in soil microbiology and development of herbicide resistant weeds. There are also concerns about unknown health effects, as it is hard to predict whether a protein will cause an allergic response. Finally, there are concerns that the use of genetic modifications gives large commercial agribusinesses an unfair advantage over small family farmers. On the other side, the use of genetically modified foods allows for the development of more nutritious and disease resistant foods; without genetic modifications, papayas would be extinct, and the development of rice strains that produce vitamin A have the potential to eliminate blindness in developing countries. The use of genetic modifications, especially herbicide resistance and insecticidal genetic modifications, have allowed for much larger scale farming, increasing the overall productivity of farms and allowing us to produce enough food for a growing global population.

Discussion Questions

1. What are your initial reactions to this option?

2. Should the decision to use GM foods be a personal or national decision?

3. Do the potential benefits of genetically modified foods outweigh the potential problems?

4. Do you trust the government to properly ban or allow certain foods? Why?

5. By banning GM foods, what are we accomplishing? What are we sacrificing?