



Conceptual Article

Development of interactive PowerPoint simulations as part of an online laboratory on the construction and identification of a genetically modified organism for non-science majors

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During the COVID-19 pandemic, on-ground laboratory classes switched to online learning. Many of these online laboratory activities focused less on laboratory techniques and more on data analysis. We developed an online laboratory activity for non-science majors focusing on the topic of genetically modified organisms. To allow online students the experience of learning first-hand techniques that they would have learned in the lab, we have designed interactive PowerPoint simulations covering the techniques of gel electrophoresis, constructing various GMOs and enzyme linked immunosorbent assay. Students complete each of these activities in order to collect data and determine whether a particular plant has undergone genetic modification. These PowerPoints are modifiable to meet the specific needs of a particular course or lesson.

Keywords: Genetically modified organisms, online laboratory activity, non-science majors, climate change, ELISA

1. Introduction

Although laboratory courses may vary in size, structure, and pedagogical content, the primary purposes are to engage students in active learning to help understand concepts from lectures (McClanahan & McClanahan, 2002) and to build the relationship between lecture and practical activities (Adams, 1998). This provides for experimental confirmation of lecture topics, which makes them more relevant and interesting than memorization of facts and ideas presented by a professor or textbook. Because lab courses have smaller class sizes, there is greater opportunity for instructors to tailor activities toward a more positive experience for students.

Building on lecture concepts and improving the science literacy of non-science majors are two important goals for laboratory instruction (Nastase & Scharmann, 1991). When properly structured, lab courses allow students to relate prior knowledge or past experiences to provide more familiarity with the subject matter. As a result, laboratory instruction may appeal to student interests and motivation, especially if presented using a local or community context (Adams, 1998).

In this paper, we describe an interactive approach to engage non-science majors taking an introductory environmental science lecture-lab course. Lessons in laboratory courses exist on a spectrum from “cookbook”-style experiments with spelled-out procedures to inquiry-based instruction where students may direct their learning based on their own interests. Because non-science majors taking lab courses do not have the depth or breadth of exposure as students majoring in the sciences, it is often beneficial to provide structure to the lab, leading students to “discover” the answer on their own, which leads to greater retention of the concepts being covered in the course (Adams, 1998).

One topic covered in our environmental science course is genetically modified organisms [GMOs] in relation to climate change and sustainable agriculture. GMOs are considered one mechanism of reducing the impact of global climate change (Kapoor, 2022). They can grow under biotic and abiotic stresses and can help achieve food security. GMOs have experienced alterations to their genomes to enhance existing traits or to introduce a trait that otherwise would not naturally occur in the species (Kumar et al., 2020). This modification can occur through selective breeding, plant grafting and through the use of genetic engineering. Genetic engineering is the addition, removal or modification of DNA in an organism. These GMOs can also be known as genetically engineered organisms [GEOs] (Edmisten, 2016). The gene coding for the enhanced trait may be transplanted from another distinct species, including an unrelated plant, animal, fungus, bacteria, or virus. With the development of genetic engineering technologies in the 1970s, humans were able to bypass the long evolutionary process of DNA mutations being selected by the environment (National Academy of Sciences, Engineering, and Medicine, 2016). Selective breeding individuals to produce desirable traits in the following generation may also potentially promote undesirable traits. Genetic engineering allows traits to be acquired in a single generation while excluding traits that are not intended to be passed along. We teach this topic in a lab setting such that students can see how GMOs are made and what tests can be done to detect whether something is a GMO. We emphasize that not all GMOs can be easily identified by just looking at them.

GMOs have played an important role in supporting humanity's rapid population growth dating back to the agricultural revolution and the use of artificial selection to enhance desirable traits in cultivated animals and plants (Wright, 2005). In the 21st century, the progress surrounding GMOs has continued to advance while many other technologically based science fields have slowed. The development of the Clustered Regularly Interspaced Short Palindromic Repeats [CRISPR] sequences for removing, replacing, and installing desired genetic sequences is at the forefront of scientific advancements (Redman et al., 2016). This technology has already proved itself to not only be relevant to agriculture and human health through the lens of environmental science right now but may become even more so in the decades to follow. Our lesson demystifies the science and rumors surrounding GMOs.

The effects and mechanisms of climate change affecting our species and others are a critical focus of our lab curriculum. This GMO lesson allows us to tie climate change to geneticists' efforts to engineer species that are well equipped to survive the changing climates around the planet. For example, we are able to reference the drought conditions causing desertification that are pushing on the natural range of agricultural species that are critical to the food supply, like rice and canola, (Zhao et al., 2006). Students then explore how modified versions of these species are showing promise to convey drought resistance and lower water consumption needs while still producing high yields (Liang, 2016). This exercise exemplifies the concept of sustainability in that we can meet our present needs without compromising the needs of future generations. Similarly relevant are the traits of disease- and pesticide-resistance that permit us to reduce adding contaminants of concern into the natural environment with the hope of avoiding more emerging contaminant threats such as what we are seeing now with per- and polyfluoroalkyl substances [PFAS] (Richardson & Kimura, 2020). Including these genetic modifications in this lesson allows us to expand upon other science topics that are critical to an understanding of modern environmental issues.

Prior to the COVID-19 pandemic, students completed a laboratory activity on GMOs whereby they first learned how to isolate and visualize DNA, then how to make a simple GMO by transforming competent *Escherichia coli* cells with pGLO (Deutch, 2019). pGLO is a plasmid designed by Bio-Rad laboratories that contains the gene that encodes for green fluorescent protein [*gfp*], under the control of an arabinose positive inducible promoter. The plasmid also contains the gene that confers resistance to ampicillin. Identifying this GMO is rather simple since the changed trait is one that can be easily observed. *E. coli* transformed with pGLO will glow green when plated on nutrient agar containing ampicillin and arabinose and exposed to ultraviolet

light. Students were then tasked with identifying a plant that is Round-Up® Ready. Round-Up® Ready plants are resistant to glyphosate, an herbicide and active ingredient in Round-Up® (Barry et al., 1997; Padgett et al., 1995). Unless a plant has already been sprayed with Round-Up, it is difficult to visually identify the plant that has this genetic modification. Therefore, students use different techniques to identify which plant is Round-Up® Ready.

The first method of identification is the Enzyme Linked Immunosorbent Assay (ELISA) (Clark et al., 1986). This technique relies upon the specificity of antibodies to recognize and bind to specific epitopes of antigens. There are three versions of ELISA. In the direct ELISA, antigens are plated into the wells of a microtiter dish and an antibody that is conjugated to an enzyme is added. If the antigen is present in the sample, the antibody will bind to the antigen. The wells are washed to remove any unbound antibodies. The presence of an antibody is then detected by the addition of a substrate that reacts with the enzyme. The indirect ELISA relies on two antibodies. A primary antibody recognizes the antigen of interest while a secondary antibody, conjugated with an enzyme, recognizes the primary antibody. The wash steps are utilized after the addition of each antibody. The third version is the sandwich ELISA. In this technique, an antibody that detects the antigen of interest is first plated into wells. The antigen sample is then added. Finally, a secondary antibody that is conjugated with an enzyme and can recognize the antigen is added.

The second method of identification is the Polymerase Chain Reaction [PCR] (Mullis, 1987; Saiki et al., 1988). This technique amplifies fragments of DNA using a thermostable polymerase (*Taq* DNA polymerase), deoxyribonucleotide triphosphates [dNTPs] and primers that are able to bind upstream and downstream from the DNA fragment of interest. While PCR is used to amplify DNA, it is also used as a detection method. Amplification can only occur if the original template contains the DNA that the primers can recognize. A PCR product is not detected if the primers are unable to bind to their target sequence. The detected DNA from PCR can then be visualized using agarose gel electrophoresis (Lee et al., 2012).

The COVID-19 pandemic resulted in many science labs switching to an online format. This switch did allow some science classes to focus more on data analysis than specific lab techniques (Buchberger et al., 2020; Delgado et al., 2020). There have been publications recently on how to make online lab classes more interactive (Chandrasekaran, 2020; Gewin, 2020; Ray & Srivastava, 2020) including the use of take-home kits available from most science supply companies. However, the challenge we faced was that these kits did not have a specific lab on GMOs that covered all of these procedures. Some supply company kits do have DNA isolation, bacterial transformation and virtual PCR lessons, but not in the context of GMOs. To this end, we designed an online version of our GMO lab. To make the lab interactive, we developed several interactive PowerPoint slideshows where students are actively engaged in developing a GMO and perform simulations of both gel electrophoresis and ELISA. In this paper, we discuss how these interactive simulations were developed and how we have incorporated them into an online GMO detection lab.

2. Construction of Interactive PowerPoints

We have developed three different Microsoft PowerPoint simulations for our online GMO lab utilizing the 2019 version of PowerPoint. They cover the topics of gel electrophoresis, construction of a GMO using genetic engineering and ELISA. In all of the simulations, students are presented with a menu of steps (see Figure 1). Users must click the correct step in order for the simulation to continue. If an incorrect step is chosen, the user is notified and allowed to try again. The user is also notified if they have selected a step that already has been completed. This has been achieved by using PowerPoint's hyperlink feature (in document) to skip to particular slides depending upon the student selection. Once the PowerPoint file is written, it is saved both as a PowerPoint presentation (.pptx) and as a macro-enabled show (.ppsm).

Unlike pre-made lab simulations that can be purchased from lab supply companies, the benefit of using interactive PowerPoints is that it allows the student to think their way through the

simulation, rather than just clicking each step and seeing the final result. Pre-made lab simulations are usually not editable. The .pptx file is available for instructors to modify and edit. By using PowerPoint simulators, instructors can modify each procedure to the level of detail they think is appropriate for their students. The GMO construction simulation can be modified for any specific GMO an instructor wishes their students to learn.

The .ppsm file is the file presented to the students to use. This file immediately opens as a slide show. This way, the students must work their way through the show to complete the activity rather than just skipping to the end. To ensure that students do not move the slides by just clicking with their mouse or pressing “enter” or “>” on the keyboard, the slide show is set in “Kiosk mode”. Kiosk mode can be set by going to the “Slide Show” menu and selecting “Set Up Show.” This way, the only way you can advance the slide is by selecting the correct step in the presentation. These simulations can be found at https://osf.io/pg4u9/?view_only=9f6c4546b5264a7c820ffd482d15743d. Both the slide show and an editable PowerPoint file are provided.

Figure 1

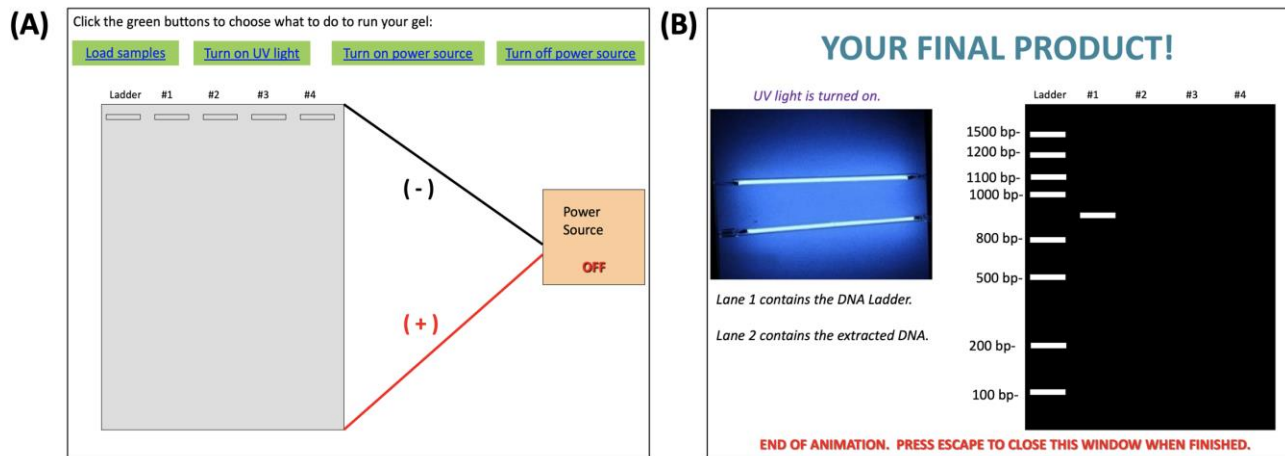
Slide deck of PowerPoint simulation



Note. Slides contain hyperlinked text or arrows to allow users to either move ahead or receive a notification that the option chosen was not correct. Slides indicating that a step has already been performed are also included.

In the gel electrophoresis simulation (see Figure 2), students are presented with a DNA ladder and a sample of DNA already placed in loading buffer. The loading buffer is used to help sink the DNA sample into the wells of the gel. The loading dye consists of a dye and glycerol. The gel itself is made of agarose, which acts as a molecular sieve to separate DNA molecules according to size. The gel contains a nucleic acid stain such that DNA can be observed. Two common stains are ethidium bromide or SYBR-SAFE. Users using the simulation must first load their samples into the gel. They must then turn on a power source for electrophoresis to commence. Because DNA is negatively charged, separation of DNA molecules is achieved by electricity. The DNA is loaded at the negative end of the gel. When the power is turned on, it will migrate towards the positive end. In the simulation, students observe the loading dye moving through the gel. Once electrophoresis has been completed and the loading dye has moved down the gel, students must turn off the power source and then use ultraviolet light to visualize the DNA, which is now visible due to ethidium bromide/SYBR-SAFE. As seen in Figure 2, students see that the DNA sample they loaded measures approximately 900 bp.

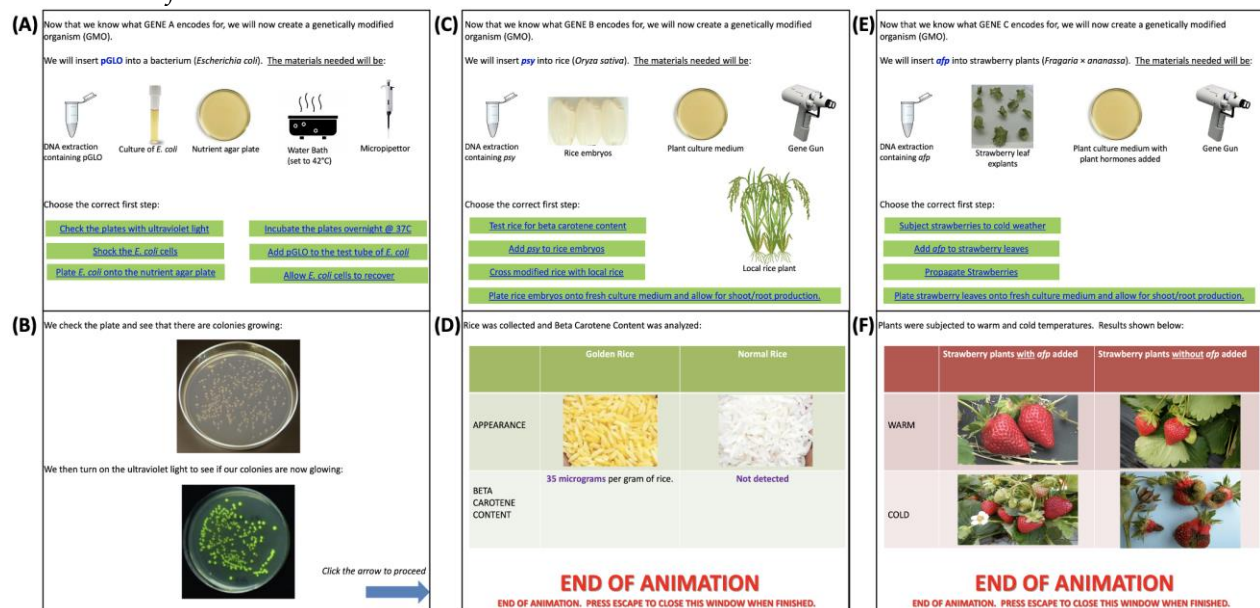
Figure 2
Screenshots of the Gel Electrophoresis Simulation



Note. (A) A gel connected to a power source is shown. Students must select the correct steps in order to load, run and visualize the gel. (B) After turning on the ultraviolet light, students can see the size of extracted DNA.

In the GMO construction simulation (see Figure 3), students are first given one of three gene sequences (see Table 1). Students must first use the Basic Local Alignment Search Tool [BLAST] (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al., 1990). By entering a gene sequence into nucleotide BLAST’s search query, users can identify the name of the gene, where it was found and for what the gene encodes. The students then are tasked with constructing a GMO by adding their assigned gene to the cells of a particular organism. In one simulation, students see that the addition of green fluorescent protein to *E. coli* results in bacterial colonies that glow in the presence of ultraviolet light. In a second simulation, students see that the addition of phytoene synthase results in the production of beta-carotene, a precursor to Vitamin A. This is known as Golden Rice. Finally, the third simulation has an antifreeze gene added to strawberries, resulting in frost-hardy strawberries.

Figure 3
Screenshots of the GMO Construction Simulation



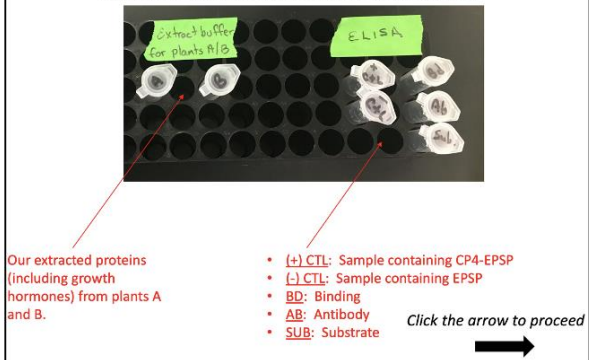
Note. (A/B) Students can add pGLO containing *gfp* to competent *E. coli* to see that colonies glow in the presence of ultraviolet light. (C/D) Students can add *psy* to rice to produce Golden Rice with higher beta carotene content. (E/F) Students can add *afp* to strawberry leaves to produce frost-hardy strawberries.

In the ELISA simulation (see Figure 4), students are presented with two different plants. One of the plants contains normal growth hormone (EPSP). Plants with EPSP are sensitive to glyphosate. The other plant is Round-Up® Ready. Round-Up® ready plants are genetically modified to produce a different growth hormone, CP4-EPSP, which is resistant to glyphosate (Barry et al., 1997; Padgett et al., 1995). The students begin with collecting a sample of each plant and grinding them in an extraction buffer to isolate plant proteins, including any growth hormones. After collecting their sample, students are presented with a positive protein control sample that contains CP4-EPSP, a negative protein control sample that contains EPSP, binding buffer, wash buffer, enzyme-linked antibodies that detect CP4-EPSP and substrate reagent. Students must first add the binding buffer to the wells of a microtiter plate, then add their protein samples and the antibody. After adding the antibody, students must wash the wells to remove any unbound antibody. Finally, the substrate is added to the wells. Any wells that contain CP4-EPSP will have enzyme-linked antibodies present in them. The addition of the substrate causes a colorimetric reaction. As seen in Figure 4, the result of the simulation shows that both the positive control and plant A wells turned red, indicating that these samples did in fact contain CP4-EPSP.

Figure 4
Screenshots of the ELISA Simulation

(A) Now that we have our extracted proteins from Plant A and B, we can use ELISA to determine which plant has the modified growth hormone (CP4-EPSP).

Our other materials we need to complete an ELISA are:



- **(+) CTL:** Sample containing CP4-EPSP
- **(-) CTL:** Sample containing EPSP
- **BD:** Binding
- **AB:** Antibody
- **SUB:** Substrate

Click the arrow to proceed

(B) Click the green buttons to choose what to add to your wells:

Add Samples to wells Add Binding Buffer to wells Add Substrate to wells Add Antibody for CP4-EPSP to wells

Wash wells

ELISA Plate:

(+) Ctl			(-) Ctl			Plant A		Plant B		
1	2	3	4	5	6	7	8	9	10	11
○	○	○	○	○	○	○	○	○	○	○

(C) YOUR ELISA RESULTS:

ELISA Plate:

(+) Ctl			(-) Ctl			Plant A		Plant B		
1	2	3	4	5	6	7	8	9	10	11
●	●	○	●	○	○	●	●	○	○	○

END OF ANIMATION. PRESS ESCAPE TO CLOSE THIS WINDOW WHEN FINISHED.

Note. (A) The materials available to the students to use are shown. (B) Students are then tasked with selecting the correct steps in order to complete the ELISA. (C) Screenshot showing the results of the ELISA.

3. Summary of Online Laboratory Lesson Using these Simulations

3.1. Discussion of the Pre-Lab

Prior to students going through the online lesson (Appendix), instructors should give a pre-lab lecture introducing the topic of GMOs. Instructors should begin with a review of the structure of DNA, emphasizing that genes dictate the traits that an organism expresses. Through genetic

modification, we can select for the most desirable traits in certain species. At this point, the DNA precipitation activity utilizing at-home materials is explained. Following this, we cover the basics of gel electrophoresis and why it is a useful tool to visualize DNA. We conclude our initial discussion of DNA by explaining how the BLAST website works (Altschul et al., 1990) and it can be used to identify a sequence of DNA.

The second topic that should be covered in the pre-lab is the concept of genetic engineering and some of the techniques that are used in laboratory settings to achieve these changes. These can include heat or electrical shocking, gene guns, and micropipettes. Explaining the concept of competence in bacterial cells helps drive home that not all genetic modifications happen through human intervention and that this can also be naturally occurring. To enforce the practical uses of these technologies, a few historic examples are provided to students of how GMOs have been used to solve issues such as the use of herbicides on crops (Round-Up® Ready soy), natural pests to crops (Bt Corn) (He et al., 2003), and food supply shortages (Aqua Advantage salmon) (Entis, 1998; Waltz, 2016). The technique of shocking was used to create Round-Up resistant soy plants. This technique is explained and in doing so references the particular growth hormone changed (CP4-EPSP) that confers this new resistance trait. Students are questioned on what physical changes are noticeable between the seeds and plants of the GMO and non-GMO versions of the species where they learn that genetic changes do not always have to be physically manifested. Without the addition of Round-Up to the two plants, they and their seeds are practically identical. It is at this point that the Enzyme Linked Immunosorbent Assay [ELISA] test is described. We emphasize to our students that ELISA is also used in other tests, such as pregnancy tests and the rapid COVID tests.

The final topic covered in the lecture is the concept of using PCR to amplify and detect specific sequences of DNA and then compare it against known samples for desired traits. Most students have heard of PCR before given that it is another COVID test.

3.2. Materials Needed

The first part of the lesson asks students to isolate DNA by taking a sample from their own cheek cells using a saltwater solution. Materials typically needed for DNA isolation include either shampoo or dish detergent, salt, water and alcohol (either ethanol or isopropyl). The remaining activities of the lab can be completed on a computer. Students should have access to Microsoft Office as the simulations run through PowerPoint.

3.3. Activity and Results

A copy of the full online activity for students and teachers can be found in the Appendix. Because the construction of a GMO involves introducing DNA, we begin by having students isolate DNA from their own cheek cells. To do this, students briefly gargle a saltwater solution and then spit into a container. A drop of dish detergent is then added to break open the cheek cells, followed by isopropanol to precipitate the DNA out of solution. Students observe the DNA to be a slimy, stringy opaque polymer. Students are then shown that we can utilize gel electrophoresis to visualize DNA by working through the gel electrophoresis simulation.

Once students have learned about isolating and visualizing DNA, they move on to the second portion where they first learn about how scientists can use the BLAST program to identify sequences of DNA. Students are assigned one of three possible gene sequences (Table 1). Using BLAST, students can discover from where this gene sequence was isolated and for what the gene encodes. This allows students to make predictions about what potential change in traits will occur when this gene sequence is introduced into a new organism. For this activity, students work through the GMO Construction simulation. Following the activity, students are asked to summarize how their GMO was made.

For the third activity, students are presented with an image of two plants. They are told that one of them is genetically modified to be Round-Up Ready. The students are then challenged to determine which plant is the GMO. Students are first asked to make visual observations of the

plant and seed. It is important to note that the pictures show plants and seeds that have not been sprayed with Round-Up. In our experience, students try to find some difference between the plants and seeds in order to identify the GMO. If a plant looks bigger or healthier, they will generally assume that the plant is genetically modified. These visual traits however are not what have been genetically modified. It is only their resistance to glyphosate. Hence, examining plants and seeds that have not been sprayed with Round-Up does not help in identifying which is genetically modified. Students then work through the ELISA simulation to find that plant A has CP4-EPSP and therefore is the GMO.

Students then confirm their ELISA simulation results by examining simulated PCR results (Appendix). A primer that recognizes tubulin is used as a positive control to verify that plant DNA is detected. A second primer that recognizes the 35S promoter used in expressing any introduced modified gene is used as the test reaction to see if any foreign DNA is present. As seen in the appendix, a PCR product is detected with both the plant and 35S primers for plant A. This result is in agreement with the ELISA simulation. In plant B, a PCR product is detected only with the plant primer, confirming that plant DNA was used in the reaction but does not show any genetic modification. As a final confirmation of their results, students are then shown pictures of the plants after being sprayed with Round-Up. As expected, plant A survives.

4. Conclusion

This lesson was one of the most successful changes we made to our curriculum during the COVID-19 remote teaching phase of this course. Not only did it allow students to experience an interactive lab while at home, but it allowed them to engage with the concept of GMOs using practical examples. GMO technology is becoming more prolific in its use and application around the world, so it is important for students to grasp this concept and destigmatize it through learning. The use of making our own PowerPoint simulations rather than pre-made lab simulations allowed us to tailor experimental simulations to the level of detail appropriate for our class and use examples that fit our curriculum perfectly.

The responses we have received from students following this lesson have been positive and some recognized the work that went into designing this module specifically for this class and situation. Prior to using these PowerPoints and teaching our GMO lesson online, students averaged 78% on their GMO lab submission. After introducing these PowerPoints into the online assignment, students averaged 86% on their lab submission. Given the success of this lesson, its use has been expanded to our online non-majors course on heredity and has been used both in-person and online in introductory food marketing classes.

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