

Assessing the Effectiveness of a Wet-Lab PCR Simulation in Biology Education

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ABSTRACT

Polymerase chain reaction (PCR), a technique for amplifying small amounts of DNA, has revolutionized molecular biology. PCR has had a profound impact on how scientists study and manipulate DNA and consequently, it is increasingly incorporated into science curricula. The precise nature of the technique, however, can make it extremely challenging in the inexperienced hands of high school and undergraduate students. The number of steps involved, need for sterile technique, and the handling of extremely small volumes all make PCR cumbersome for students. Additionally, the noncarcinogenic electrophoresis stains such as methylene blue provide inferior visualization for analysis. Even if educators have the resources for including PCR labs into the course curriculum, ambiguous results are a deterrent. Many teachers also are hindered by the expense and time required for PCR reactions, electrophoresis, staining/destaining, and analysis. We have developed curriculum for a PCR simulation to be used in place of authentic PCR in an effort to encourage more widespread use of PCR technology in biology classrooms. The PCR simulation is designed to yield clear and consistent results with minimal manipulations and expense. The simulation was taught side-by-side with a conventional PCR kit marketed to educators. This study was completed in two undergraduate classes at Southern Illinois University Edwardsville and the effectiveness of the simulation in comparison to authentic PCR was determined by analysis of pre and post assessments.

INTRODUCTION

Polymerase chain reaction (PCR) is a technique for amplifying small amounts of DNA that has revolutionized molecular biology. Terrific scientific advances, in disciplines such as genetics, medical science, DNA forensics, and transgenic plant science have been possible using this technology since its development in 1983 (Mullis et al., 1986). Since PCR has had such a profound impact on how scientists study and manipulate DNA it has increasingly been incorporated into secondary and post-secondary science curricula. Previous studies have shown that inquiry teaching can have a positive impact on learning (Rakow, 1986) and PCR provides a valuable tool for inquiry-based learning in molecular biology laboratories. While active learning suggests students are physically participating in the lesson, inquiry learning requires that students are also mentally participating in it. The mental participation is more important than the physical participation in imparting lasting understanding (Lord and Orkwiszewski, 2006). An inquiry PCR lesson would require students to actively explore the lesson questions and objectives, manipulate the PCR reaction components, and make predictions based on their experimental results. The precise nature of PCR, however, can make it challenging in the inexperienced hands of students. The large number of steps involved, lack of proper sterile technique, and

inexperience at micropipetting make this technology cumbersome and unreliable in science classrooms. Additionally, nontoxic, noncarcinogenic, and inexpensive components are preferred for undergraduate science laboratories and necessary in secondary schools. PCR products are typically visualized following gel electrophoresis with the mutagenic stain ethidium bromide. Methylene blue is an alternative DNA stain that is noncarcinogenic but results in inferior staining and often requires more time for staining and destaining (Herrin and Schmidt, 1988). The equipment and various components necessary for authentic PCR are expensive. For example, programmable thermal cyclers for authentic PCR can cost \$2000-\$4000 for a basic unit.

Even when teachers have the financial resources and equipment to include PCR in their lessons, ambiguous results can be a major deterrent. While authentic PCR is considered quick among research scientists, it requires more time than is available in most classroom laboratory sessions. The time required for PCR reactions, gel electrophoresis, gel staining/destaining, and analysis can deter interested teachers.

In an effort to encourage more widespread inclusion of PCR in biology lab courses, we developed a curriculum for a realistic PCR wet-lab simulation to be used in the place of authentic PCR. The PCR simulation yields clear and consistent results with minimal manipulations and minimal expense. Our objectives are to teach the same lesson as authentic PCR while removing the potential for errors and ambiguous results, reducing cost, reducing teacher preparation time, reducing class time, and making PCR more accessible to biology educators.

MATERIALS AND METHODS

Authentic PCR and the PCR simulation were taught side-by-side in two separate classes over two days at Southern Illinois University Edwardsville. One class consisted of 14 students majoring in secondary science, some of whom had previous experience with PCR. The other class consisted of 11 students majoring in elementary education, none of whom had previous PCR experience. Groups of 3-4 students were randomly assigned to either the authentic PCR lesson or the PCR simulation in each class.

Participating students completed a pre-test to assess their prior knowledge of the subject matter and to self-rate their motivation as students (Figure 1). The pre-test consisted of a nine item Likert-like personal assessment, 12 multiple choice questions, and three short answer questions. After completing the pre-test, all students received a handout containing general information about PCR, a laboratory protocol, and questions for discussion. Figure 2 shows the complete handout received by groups performing the simulated PCR and Figure 3 is the laboratory protocol used by groups performing authentic PCR. We designed the laboratory protocol for the PCR simulation to resemble the authentic PCR protocol; consequently, students were not aware that there were two different curricula performed simultaneously in the classes.

The authentic PCR groups used components from the BioRad Biotechnology Explorer: Genetically Modified Organism (GMO) Investigator Kit (Mardigan and Wiseman, 2007). The GMO Investigator Kit includes reagents and instructions for extracting DNA from three sources; certified non-GMO seeds, known GMO seeds, and a test food of unknown

GMO status. However, we performed the DNA extractions in advance due to time constraints and we used non-GMO frozen corn as the test food. PCR amplified sequences for this exercise were from the 35S promoter of the cauliflower mosaic virus (CaMV 35S) and the Photosystem II chloroplast gene (PSII). CaMV 35S is a promoter commonly used in GMOs and the PSII was included to test for successful DNA extraction from plant material. PCR primers for CaMV 35S and PSII were mixed individually with the three DNA samples (GMO+ DNA, GMO- DNA, test food DNA) for a total of six PCR reactions per group (Figure 4). PCR reactions were completed according to manufacturer's recommendations. The students were able to visualize the presence or note the absence of the amplified DNA by agarose gel electrophoresis (Figure 5). Students determined whether the DNA extractions were reliable by the presence of a 455 bp PSII band in lanes 1, 3, and 5. They also determined whether or not the test food was derived from a GMO crop by the presence or absence of the 200 bp CaMV 35S band in lane 4.

Our curriculum for the simulated PCR wet lab was based on the BioRad Biotechnology Explorer: Genetically Modified Organism (GMO) Investigator Kit model (Mardigan and Wiseman, 2007). The students used the same laboratory techniques and equipment, were asked the same questions, and went through the same inquiry/discovery process as in the authentic PCR laboratory. The simulation included the same six-sample format as the BioRad Kit. However, the initial six sample tubes contained 20 μ l of water or plasmid DNA (0.5 μ g/ μ l) instead of extracted genomic DNA (Figure 4). The plasmids pUC18 (2.7 kb) and Mp18 (7.3 kb) were used because they resembled the size relationship of the CaMV35S (200 bp) and the PSII (455 bp) PCR products of the authentic PCR lesson as visualized on an agarose gel (Figure 5). These plasmids are also readily available (Fisher Scientific, USA) and inexpensive. Sample tubes 1, 3, and 5, corresponding to wells 1, 3, and 5 on the gel, contained Mp18 plasmid DNA. Sample tube 6, corresponding to well 6, contained pUC18 plasmid DNA. Sample tubes 2 and 4, corresponding to wells 2 and 4 on the gel, contained 20 μ l of water. Note that if the "test food" was to be represented as GMO positive, pUC18 plasmid DNA would be added to tube 4 instead of water .

Each group received a tube of "plant master mix" and a tube of "GMO master mix" to add to the DNA sample tubes. In the BioRad GMO Investigator Kit these tubes contained the components necessary for traditional PCR (Taq polymerase, primers, dNTPs, and buffer). The only difference between the master mixes was the primers. The "plant master mix" contained primers for amplifying the PSII gene fragment and the "GMO master mix" contained primers for amplifying the CaMV 35S fragment. The master mix tubes for the simulation kits contained 20 μ l of distilled water. Five μ l of the "plant master mix" was micropipetted into tubes 1, 3, and 5 and five μ l of the "GMO master mix" was added to tubes 2, 4, and 6.

The samples of the authentic PCR groups and the simulated PCR groups were cycled in the thermal cycler as follows: one two minute denaturation cycle at 94°C followed by 35 cycles of amplification that consisted of one minute of denaturation at 94°C, one minute annealing at 59°C, and two minutes extension at 72°C. Lastly, there was a ten minute final extension at 72°C.

PCR products were visualized using electrophoresis on 1% agarose gels. Five μ l of loading dye was added to each sample prior to loading the gel and gels were electrophoresed

for 20-30 minutes at 120 volts. Gels were then left in a 1x solution Fast Blast DNA Stain (Bio-Rad Laboratories, Hercules, CA) at 4°C for 48 hours until the next class meeting. Students examined the stained gels and noted the DNA bands in each sample to determine whether or not the test food contained GMOs.

Students completed the questions accompanying the lab handout (Figure 2) after completing all lab procedures. At the next class meeting, two days later, students were given the post-test to assess comprehension and short-term retention of the lesson. Results for authentic PCR and simulated PCR were analyzed by comparing the pre- and post-test results using two-tailed, paired t-tests.

RESULTS

Students in the simulation groups showed significant improvement ($P < 0.001$) from a pre-test mean score (\pm SE) of $40.5 \pm 6.3\%$ to a post-test mean score of $62.4 \pm 5.6\%$. Students in authentic PCR group also showed significant improvement ($P < 0.001$) from a pre-test mean of $40.6 \pm 7.8\%$ to a post test mean score of $65.5 \pm 5.5\%$. The two groups improved, by $21.9 \pm 6.1\%$ and $24.8 \pm 7.9\%$ respectively, but there is no statistical difference in learning between teaching methods ($P = 0.608$).

Science majors and nonscience majors rated themselves in two main areas before participating in the exercise: motivation as a student and prior knowledge of PCR. On a 1-5 scale, the mean motivation ratings were 4.5 for science majors and 4.0 for nonscience majors. The mean ratings for prior knowledge of PCR was 4.9 for science majors and 3.4 for nonscience majors. Given their ratings, we decided to analyze for any differences in learning between the science majors and the non-science majors. Science majors ($n = 14$) showed significant improvement from a pre-test mean score of $45.7 \pm 6.0\%$ to a post-test mean score of $69.5 \pm 5.0\%$. The nonscience majors ($n = 11$) showed significant improvement from a pre-test mean score of $33.9 \pm 9.3\%$ to a post-test mean score of $55.8 \pm 7.9\%$. While the average pre-test and post-test scores were higher for science majors compared to the nonscience majors, their amount of improvement ($23.8 \pm 5.3\%$ and $21.8 \pm 8.7\%$ respectively) did not differ ($P = 0.757$).

DISCUSSION

We have demonstrated that college students performing a simulated PCR lab showed equivalent academic gains as students performing a comparable authentic PCR lab. Additionally, science majors and nonmajors using the simulation had equivalent gains as majors and nonmajors performing authentic PCR. Incorporating the simulated PCR lesson, however, yields many benefits beyond student success. These benefits include time and money savings as well as ease of implementation for both the teacher and students. Teacher preparation time is drastically reduced from 7-8 hours with the conventional kit, to 2-3 hours with the simulation. In fact, multiple uses of the simulation take much less time to prepare since the teacher can aliquot samples for several labs at one time and store them in the freezer. Teacher preparation for the authentic PCR lesson includes thawing the master mix and primers within 30 minutes of use, an extremely difficult task for teachers with back to back laboratory sections. Warming can denature key components and timing/handling is critical to successful DNA amplification. These are not

considerations for the simulation since the reaction components are replaced with water. Simulated PCR also reduces classroom time since it does not require DNA amplification. In this investigation, students used authentic PCR and simulated PCR in the same laboratory, at the same time, which necessitated running through thermal cycles for the groups doing authentic PCR. If the simulation is taught alone, the time in the thermal cycler could be greatly reduced, or even omitted, since amplification is not necessary for visual results. Teaching what the thermal cycler does is crucial to teaching PCR, even if it is not used in the classroom for the simulation. An option for teachers without access to a thermal cycler is to use water baths with a reduced number of cycles and reduced time at each temperature to simulate the temperature cycling that takes place in authentic PCR.

Cost is also an important factor when considering labs to be included in laboratory courses. The BioRad GMO Investigator Kit costs approximately \$25.00 per group, while the simulated PCR lesson costs approximately \$6.00 per group. The cost of simulated PCR could be reduced even further for teachers who have the personal or departmental expertise to purify plasmid DNA themselves instead of purchasing plasmid DNA. This cost difference could make teaching the PCR simulation feasible for teachers with small, fixed, annual budgets.

Molecular biology concepts and laboratory techniques are increasingly being taught in high school biology courses (Stiller and Coggins, 2006). The time and financial benefits of simulated PCR over authentic PCR may encourage more teachers to incorporate lab experiences into the high school curriculum. Giving high school biology students an introduction to proper laboratory techniques and molecular biology procedures such as PCR sets them up for potentially greater success in their post-secondary academic science careers (Fernandez-Novell et al., 2002). Overall, simulated PCR lessons promise to be useful tools in post-secondary and secondary biology laboratories.

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Figure 1. Pre-test and post-test administered to all student participants prior to beginning the PCR lesson and two days after completion of the lesson.

Please circle the best answer for the questions below.

1. What is DNA?
 - a) an organism's genetic material
 - b) an organism's desmosomic material
 - c) a product of translation
 - d) all of the above

2. A specific sequence of nucleotides in DNA, the functional unit of inheritance, is known as a(n)
 - a) ELISA
 - b) gene
 - c) GMO
 - d) intron

3. What does the acronym PCR stand for?
 - a) Polymerase Chain Reagents
 - b) Polysaccharide Chelation Reaction
 - c) Polymerase Chain Reaction
 - d) Polysaccharide Chelation Reagents

4. PCR products are composed of _____.
 - a) DNA
 - b) RNA
 - c) both a & b
 - d) none of the above

5. What is a transgenic organism?
 - a) an organism that has one or more genes from another organism incorporated into its DNA
 - b) an organism that has not had any foreign genes incorporated into its DNA
 - c) a genetically modified organism
 - d) both a & c

6. Choose the ranking from most stable to least stable over long periods of time?
 - a) RNA, DNA, protein
 - b) DNA, RNA, protein
 - c) protein, RNA, DNA
 - d) DNA, protein, RNA

7. How many template strands of genetic material must be present for PCR to be successful?
 - a) 1000
 - b) 100
 - c) 1

8. How can you test food to find out if it contains material derived from a genetically modified organism (GMO)?
- UV light
 - PCR
 - scanning electron microscopy
 - both b & c
 - all of the above
9. Which of the following is **NOT** a necessary component(s) of PCR?
- polymerase
 - calcium
 - nucleotides
 - primers
 - magnesium
10. What type of molecule is polymerase?
- polysaccharide
 - nucleic acid
 - protein
 - carbohydrate
11. Primers are _____
- single stranded
 - double stranded
 - necessary for polymerase function
 - a & c
 - b & c
12. What is the purpose of the 72 degree incubation step in PCR?
- DNA strands are denatured
 - optimal temperature for polymerase
 - primers bind to single stranded DNA
 - all of the above
 - none of the above
13. Illustrate the banding pattern in an electrophoresis gel for a sample containing 3 DNA molecules of the following sizes: 8000 bp, 4000 bp, and 2000 bp. Explain your reasoning.
14. What is the purpose of including experimental controls?
15. Many foods containing genetically modified crops are highly processed. How might genetic material from whole plants differ from that of processed foods?

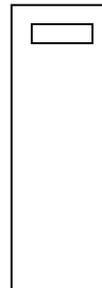


Figure 2. Laboratory handout for the simulated PCR. The handout contains general information about PCR and GMOs, the laboratory protocol, and questions for discussion.

PCR FOR INVESTIGATING GMOS IN THE LABORATORY

Adapted from BioRad Biotechnology Explorer GMO Investigator Kit #166-2500EDU

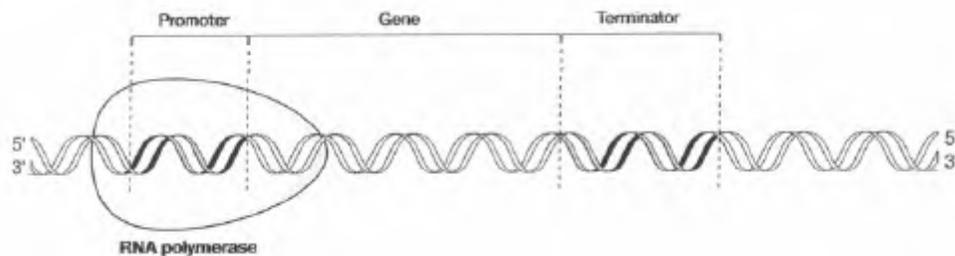
INTRODUCTION

In this lab you will use PCR to test plant extracts. Are the plants or plant products you test from genetically modified organisms (GMOs)?

What are GMOs / GM crops?

Genetically modified crops have had DNA from another organism incorporated into its DNA. The foreign genetic material usually codes for proteins that gives the plants an advantage over non-GMO plants. Examples of traits created via genetic modification include pest resistance, improved fruit yield, herbicide resistance, and increased nutritional content.

For inserting foreign genetic material to work, the host plant's cells must be able to 'read' the information so that transcription is successful. This is done by adding a promoter and a terminator.



Using promoters and terminators, scientists have engineered GMOs so that the foreign gene is continually transcribed allowing the protein to be produced throughout the plant.

What are you looking for?

You will use PCR to test for the presence of the most commonly used promoters and terminators for creating plant GMOs. Since you are testing extracts of plant material and plant products, you will also be testing for the presence of plant material. Presence of the plant protein will signify successful DNA extraction from the initial product (frozen corn, corn chips, flour, soybeans, etc.).

PCR

Remember, each cycle of PCR involves three basic steps:

1. Denaturation (high temperature, 94°C)
2. Primers anneal (low temperature, 59°C)
3. Synthesis (intermediate temperature, 72°C)

Since PCR can produce large quantities of DNA from very small amounts of starting material, contamination with unwanted DNA can be a problem. Be careful to prevent cross-contamination of samples. *Always use a new pipet tip, keep tubes tightly capped while not in use, and wear clean gloves.*

The PCR reactions will take place in a thermal cycler (PCR machine) that performs the necessary cycles of heating and cooling. The thermal cycler will be programmed to run 35 cycles.

What will you test?

You will use the following control DNA samples:

GMO positive, GMO negative, and plant positive

DNA extractions have been prepared for you ahead of time to use as unknowns.

LAB PROCEDURES

Day One: Set up PCR Reactions

1. Number 6 PCR tubes 1-6 and label with your initials. The numbers on the tubes correspond to the following tube contents:

1	20µl Non-GMO food control DNA	5 µl Plant master mix (green)
2	20µl Non-GMO food control DNA	5 µl GMO master mix (red)
3	20µl Test food DNA	5 µl Plant master mix (green)
4	20µl Test food DNA	5 µl GMO master mix (red)
5	20µl GMO positive control DNA	5 µl Plant master mix (green)
6	20µl GMO positive control DNA	5 µl GMO master mix (red)
2. Keep the tubes on ice for the remaining steps.
3. Using a new pipet tip each time, add 5 µl of the indicated master mix to each tube. Cap each tube immediately.
4. Using a new pipet tip each time, add 20 µl of the appropriate DNA to each tube as indicated by the table above. Recap tubes. Flick tubes with finger to mix.
5. With instructor's assistance, place the tubes in the thermal cycler.

Preparing for Electrophoresis of PCR Products:

When the thermal cycler is done, you have completed your PCR amplification. However, you must visualize your products to determine what you have amplified. You will use gel electrophoresis to do this. 1% agarose gels have been poured before class and are ready for you to use.

Running the Gel, Electrophoresis

1. Using a new pipet tip each time, add 5 µl loading dye to each sample and mix well. Pulse tubes in centrifuge to bring all liquid to bottom of tube after mixing. Be mindful that tubes are placed in the centrifuge in a balanced manner.

2. Using a new pipet tip each time, load the wells with each sample (30 μ l) as indicated below:

1	Sample 1	Non-GMO control w/plant primers
2	Sample 2	Non-GMO control w/GMO primers
3	Sample 3	Test food w/plant primers
4	Sample 4	Test food w/ GMO primers
5	Sample 5	GMO positive DNA w/ plant primers
6	Sample 6	GMO positive DNA w/ GMO primers

3. Run your gel for 20-30 minutes at 120 volts, but be careful not to let the dye front migrate out of the agarose gel.

Staining and Rinsing Your Gel

1. Place your gel in a stain solution provided for you. Your gel will stain over the next 48 hours. The results will be ready for you to analyze at the beginning of the next class period.

ANALYSIS OF RESULTS

Once your gel has been stained and destained you will be able to see how far the DNA molecules have migrated.

<u>Lane</u>	<u>Sample</u>	<u>Bands?</u>
1	Sample 1: Non-GMO control w/plant primers	
2	Sample 2: Non-GMO control w/ GMO primers	
3	Sample 3: Test food w/ plant primers	
4	Sample 4: Test food w/ GMO primers	
5	Sample 5: GMO positive control w/ plant primers	
6	Sample 6: GMO positive control w/ GMO primers	

Draw your gel.

QUESTIONS

1. What was your test food?
2. Did you generate the expected bands w/ GMO primers (lane 6 & maybe 4)?
3. Based on Lane 4, is your test food a GMO?
4. Did you generate bands w/ plant primers where expected (lanes 1, 3, & 5)?
5. Are you confident in your results? Why or why not?
6. How do the results of the other five PCR reactions help support (or not) the result for your test food?
7. If you were to repeat this lab what might yield better results?

Figure 3. Laboratory protocol for the authentic PCR. The handout resembles the simulated PCR protocol (Figure 2) with minor variations in pipetting volumes. Variations from the simulated protocol are indicated in bold.

LAB PROCEDURES

Day One: Set up PCR Reactions

- Number 6 PCR tubes 1-6 and label with your initials. The numbers on the tubes correspond to the following tube contents:

1	20µl Non-GMO food control DNA	20 µl Plant master mix (green)
2	20µl Non-GMO food control DNA	20 µl GMO master mix (red)
3	20µl Test food DNA	20 µl Plant master mix (green)
4	20µl Test food DNA	20 µl GMO master mix (red)
5	20µl GMO positive control DNA	20 µl Plant master mix (green)
6	20µl GMO positive control DNA	20 µl GMO master mix (red)
- Keep the tubes on ice for the remaining steps.
- Using a new pipet tip each time, add **20** µl of the indicated master mix to each tube. Cap each tube.
- Using a new pipet tip each time, add 20 µl of the appropriate DNA to each tube as indicated by the table above. Recap tubes. Flick tubes with finger to mix.
- With instructor's assistance, place the tubes in the thermal cycler.

PREPARING FOR ELECTROPHORESIS OF PCR PRODUCTS

When the thermal cycler is done, you have completed your PCR amplification. However, you must visualize your products to determine what you have amplified. You will use gel electrophoresis to do this. 1% agarose gels have been poured before class and are ready to use.

RUNNING THE GEL, ELECTROPHORESIS

- Using a new pipet tip each time, add **10 µl** loading dye to each sample and mix well. Pulse tubes in centrifuge to bring all liquid to bottom of tube after mixing. Be mindful that tubes are placed in the centrifuge in a balanced manner.
- Using a new pipet tip each time, load the wells with **20 µl** of each sample as indicated below:

1	Sample 1	Non-GMO control w/plant primers
2	Sample 2	Non-GMO control w/GMO primers
3	Sample 3	Test food w/plant primers
4	Sample 4	Test food w/ GMO primers
5	Sample 5	GMO positive DNA w/ plant primers
6	Sample 6	GMO positive DNA w/ GMO primers
- Run your gel for 20-30 minutes at 100 volts, but be careful not to let the dye front migrate out of the agarose gel.

Figure 4. Depiction of the actual reagents added to the six PCR reaction tubes in for the authentic PCR (4a) and simulated PCR procedures (4b). Figure 4a shows that in authentic PCR, purified genomic DNA was added to the tubes along with Plant Master Mix or GMO Master Mix. The master mixes contained the necessary reagents for PCR amplification (Taq Polymerase, dNTPs, primers, and reaction buffer). Figure 4b shows that plasmid DNA or water was added to the tubes in the simulation protocol. Instead of adding Plant Master Mix or GMO Master Mix to the reaction tubes, the students added water.

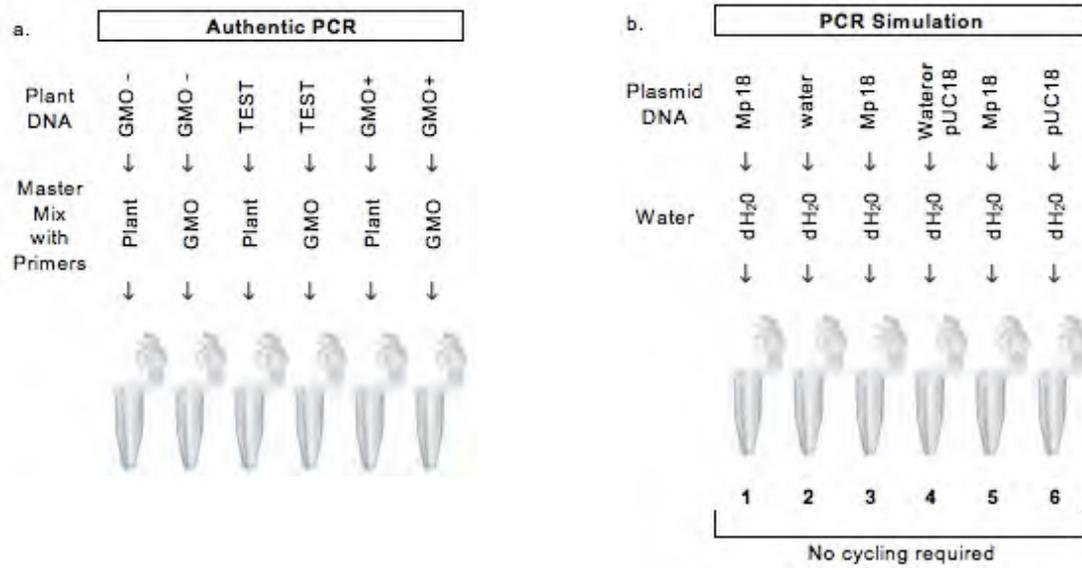


Figure 5. Diagram representing the gel banding pattern obtained for a GMO positive (left) and GMO negative (right) test food. The PCR products from the PSII chloroplast gene (455 bp) in lanes 1, 3, and 5 indicate successful DNA extractions from samples. The PCR reactions from the CaMV35S promoter (lanes 2, 4, and 6) produce a 200 bp band in lane 6 (GMO+), and no band in lane 2 (GMO-). Presence or absence of the product in lane 4 indicates whether or not the test food is from GMO plant material. The bands in the simulation gels follow the same banding pattern.

