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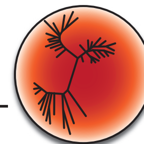
Curriculum



# Who Scared the Cat? A Molecular Crime Scene Investigation Laboratory Exercise †

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**This introductory laboratory exercise gives first-year life science majors or nonmajors an opportunity to gain knowledge and experience in basic bioinformatics and molecular biology laboratory techniques and analysis in the context of a mock crime scene investigation. In this laboratory, students determine if a human (Lady) or dog (Kona) committed the fictional crime of scaring a cat. Students begin by performing *in silico* PCR using provided dog- and human-specific PCR primers to determine the sequences to be amplified and predict PCR amplicon sizes. They then BLAST (Basic Local Alignment Search Tool) the *in silico* PCR results to confirm that the PCR primers are designed to amplify genomic fragments of the cardiac actin gene in both dogs and humans. Finally, they use DNA quantification techniques, PCR, and agarose gel electrophoresis to identify the culprit and they confirm results by analyzing Sanger sequencing. Student learning gains were demonstrated by successful execution of the lab and by analysis and interpretation of data in the completion of laboratory reports. The student learning gains were also demonstrated by increased performance on a post-laboratory assessment compared to the pre-assessment. A post-activity assessment also revealed that students perceived gains in the skills and conceptual knowledge associated with the student learning outcomes. Finally, assessment of this introductory molecular biology and bioinformatics activity reveals that it allows first-year students to develop higher-order data analysis and interpretation skills.**

## INTRODUCTION

Advancing students' technical laboratory and data analysis skills are integral objectives of life science education. Specifically, the *Vision and Change* report from the American Association for the Advancement of Science lists analytical and technical skills as core competencies for biology students (2). Development of these competencies allows students to advance their conceptual knowledge and attitudes about the field of biology.

Techniques valued in introductory labs to develop these competencies include polymerase chain reaction (PCR) and agarose gel electrophoresis. Further, given the increasing popularity of bioinformatics (i.e., identifying and predicting DNA sequences) within the field of molecular biology, advancing students' bioinformatics competencies has also been highlighted (2, 5). Numerous laboratory activities or courses have been developed to advance students' technical laboratory and data analysis skills associated with these techniques, including a few crime scene investigations (3, 7, 10, 11). For example, a semester-long nonmajors cell biology course incorporated the following forensic laboratory techniques: forensic hair analysis, phenolphthalein blood testing, fingerprinting, and PCR (3). Other advanced crime scene investigation laboratories involve the design and/or implementation of more complicated introductory techniques, such as DNA isolation, advanced PCR methods, gel extraction of DNA, and sophisticated bioinformatics analyses (7, 10, 11). These previous crime scene investigation laboratories are either the focus of an entire course or involve techniques that may be too advanced for novice students. Furthermore, while student perceptions and attitudes were assessed in these activities and were shown to have grown (3, 7), assessment of students' critical thinking or ability to analyze and interpret data was minimal.

In Who Scared the Cat?, students determine if a dog (Kona) or human (Lady) scared a cat in a fictional crime. Students solve the crime using bioinformatics techniques (*in silico* PCR and BLAST), DNA spectrophotometry, PCR, agarose gel electrophoresis, and Sanger sequencing. Our activity is unique in that it is geared towards novice students

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and provides exposure to introductory molecular biology and bioinformatics techniques. Our short activity is modular and easily fits within the constraints of existing introductory life science courses. Through completion of a lab report at the conclusion of the activity, students' ability to analyze and interpret experimental data is assessed, something which was lacking in many of the crime scene investigation activities noted above. Finally, our activity is based on a real crime scene investigation, where the molecular biology techniques used helped to determine if evidence found at an actual crime scene was of dog or human origin. Thus, our exciting and engaging activity allows first-year students to develop technical laboratory skills and analyze and interpret data in the context of solving a problem.

### Intended audience

The intended audience for this activity is first-year undergraduate students, inclusive of both life science majors and nonmajors. This activity would be appropriate in an introductory biology or biotechnology course.

### Prerequisite student knowledge

Students will need to be able to use the Internet and word processing software (e.g., Microsoft Word or Google Docs). Students will be exposed to the basic molecular biology knowledge needed to perform this activity through the lectures presented in Appendix 1. The basic laboratory skills students should have prior to the start of the laboratory component of this activity include proper use of micropipettes and balances.

### Learning time

This activity has three lectures, each being 30 to 60 minutes in length (Appendix 1). Each lecture contains clicker questions or computer activities and all three lectures should be delivered before starting the laboratory component of the activity.

The laboratory component consists of five parts. It is recommended that these be completed over the course of three three-hour laboratory sessions, although the activity was designed to be implemented by instructors according to the timing constraints of individual lab sessions. See Figure 1 for a flowchart that shows the recommended grouping and timing of each part of the lab.

### Learning objectives

Upon completion of this activity, students will be able to:

1. Describe how PCR, agarose gel electrophoresis, and DNA sequencing work
2. Predict PCR amplicon size and sequence using *in silico* PCR and BLAST
3. Evaluate the concentration and purity of DNA
4. Interpret PCR results using agarose gel electrophoresis
5. Analyze sequencing results using BLAST

## PROCEDURE

### Materials

For the lecture component of this activity, students will need access to clickers and computers with Internet access and word processing capabilities (Microsoft Word or Google Docs).

Standard molecular biology supplies will be needed for the laboratory component of this activity. The following equipment is required: spectrophotometer capable of DNA detection (NanoDrop 2000 is recommended, although any UV spectrophotometer would be sufficient), PCR thermocycler, agarose gel electrophoresis equipment (gel boxes, casting trays, power supplies, gel imager), microwave, and balance. The genomic DNA used in this activity can be purchased commercially and additional reagents include PCR primers (4), PCR master mix, agarose, molecular weight DNA ladder, and GelRed. Please refer to the Instructor Materials (Appendix 2) for a complete list of equipment, supplies, and reagents needed for this activity.

### Student instructions

Students should read the Student Handout (Appendix 3) before the lab. The Student Handout provides a summary of the crime, background information about techniques, the laboratory procedure, and discussion questions.

In part 1 of the laboratory activity, students use *in silico* PCR (8), a bioinformatics tool to predict PCR amplicon sizes and sequences based on designed primers, such as the dog or human primers provided. To perform *in silico* PCR, we recommend using the UCSC Genome Bioinformatics site, which can be accessed at https://genome.ucsc.edu/cgi-bin/hgPcr. Students then BLAST (1) the *in silico* PCR results and determine that the primers are designed to amplify fragments of the cardiac actin gene from dogs and humans (Appendix 2). BLAST can be accessed at https://blast.ncbi.nlm.nih.gov/Blast.cgi.

In part 2, students are given three genomic DNA samples: Kona control, Lady control, and crime scene. Students determine the concentration and purity of the DNA via spectrophotometry and dilute the DNA for PCR. In part 3, students use two PCR master mixes to set up four PCR reactions: Kona DNA in dog master mix, crime scene DNA in dog master mix, Lady DNA in human master mix, and crime scene DNA in human master mix. In part 4, students analyze the PCR by agarose gel electrophoresis and confirm that the dog amplicon is 275 base pairs (bp) and the human is 397 bp, which was expected from *in silico* PCR. Students are able to determine who committed the crime by comparing the agarose gel electrophoresis results from the crime scene

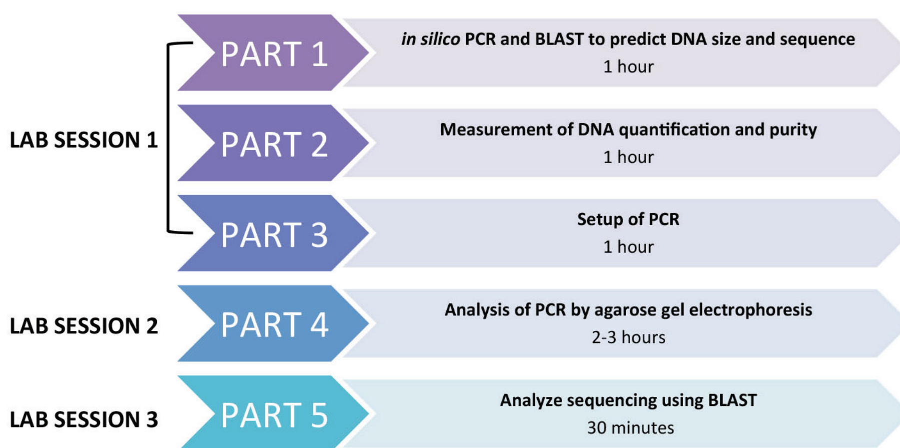


FIGURE 1. Flowchart for the crime scene laboratory activity. These are the five parts to this crime scene lab, with the approximate timing of each. It is recommended that the activity span three 3-hour laboratory sessions, with parts 1, 2, and 3 performed in session 1, part 4 in session 2, and part 5 in session 3. PCR = polymerase chain reaction; DNA = deoxyribonucleic acid; BLAST = basic local alignment search tool.

to the Kona and Lady controls. Refer to Figure 2 for representative agarose gel electrophoresis results where Lady (human) committed the crime. In part 5, students analyze Sanger sequencing data from the PCR products using BLAST to confirm who committed the crime. The complete student procedure, including discussion questions to be answered at the conclusion of each component of the activity, can be found in the Student Handout (Appendix 3).

### Faculty instructions

Refer to Appendix 1 for three lectures that cover the technical concepts involved in this activity, specifically:

1. DNA structure, agarose gel electrophoresis, and DNA spectrophotometry
2. PCR and Sanger sequencing
3. *in silico* PCR and BLAST

All three lectures should be given before the start of the laboratory components of the activity. Refer to the final slides of the lecture materials for clicker questions and a corresponding answer key.

This exercise is designed for students working individually or in pairs. Figure 1 depicts the flow of the five components of the laboratory, with an approximate timing for each. While designed to fit various course schedules, it is recommended that this activity span three 3-hour sessions. Support from teaching assistants is also highly recommended. Teaching assistants are helpful in the setup of the laboratory and in the preparation of the reagents, particularly the PCR master mixes, which are prepared while students are quantifying DNA on a spectrophotometer and may need assistance from the instructor (as outlined in Appendix 2). Teaching assistants can also be of help to students as they perform the various aspects of the laboratory.

Before the lab, the instructor must prepare reagents and the commercially available genomic DNA samples (Lady control, Kona control, and crime scene). For the crime scene sample, instructors choose who committed the crime: Lady (human) or Kona (dog). Details of reagent vendor information and preparation, equipment and supply setup, flow of laboratory events from the instructor's perspective, and a discussion question key can be found in the Instructor Materials (Appendix 2).

Two sets of sequencing data (one per potential culprit) are available in Appendix 4 for students to analyze.

### Suggestions for determining student learning

Student learning can be assessed through multiple mechanisms. First, students can respond to a series of discussion questions found at the end of the Student Handout (Appendix 3). The discussion questions are designed to get students to think critically about their data, procedural steps, and/or sources of error. Students should respond to the appropriate discussion questions at the conclusion of each component of the laboratory activity, both in writing and during a class discussion led by the instructor. Students should also include answers to discussion questions in the discussion section of their laboratory reports. Refer to page 11 of the Instructor Materials (Appendix 2) for anticipated answers to these discussion questions.

Students can also individually write laboratory reports describing the procedural steps, results, and interpretation of their crime scene investigation. Lab reports are a great way to assess all student learning outcomes for this activity. Furthermore, completion of lab reports allows students to advance their scientific writing and ability to communicate science, which are additional competencies outlined in the *Vision and Change* report (2). Laboratory report guidelines with a grading rubric (which should be provided to students)

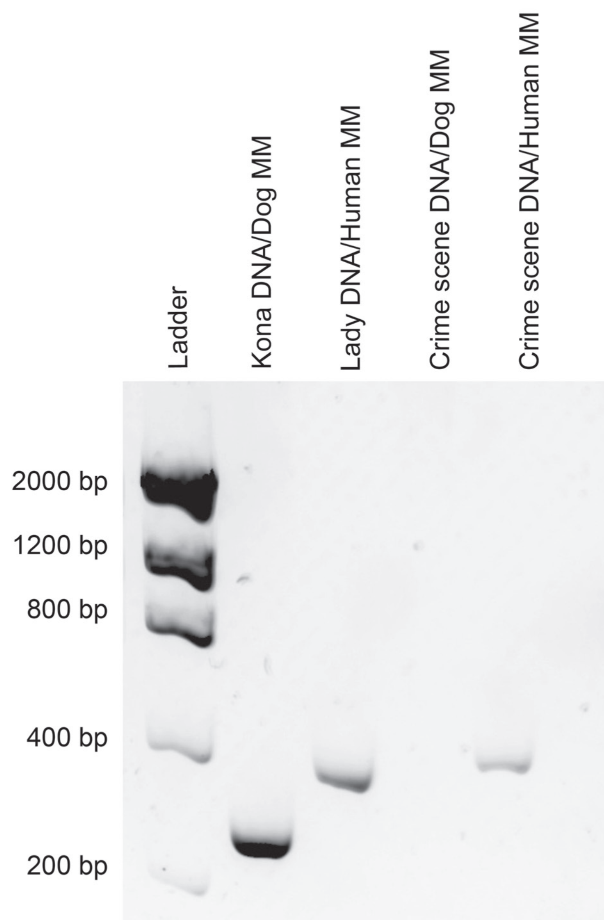


FIGURE 2. Representative agarose gel electrophoresis results from a student PCR. The sizes (base pairs; bp) of the low DNA mass ladder are depicted on the left. The samples were loaded in the following order (left to right): low DNA mass ladder (ladder), Kona control DNA in dog PCR master mix (MM), Lady control DNA in human MM, crime scene DNA in dog MM, crime scene DNA in human MM. Expected dog and human PCR amplicons are observed in the Kona control (275 bp) and Lady control (397 bp) samples, respectively. This gel depicts Lady (human) as the culprit, as there is an approximate 400 bp band in the crime scene sample in human MM. PCR = polymerase chain reaction; DNA = deoxyribonucleic acid; MM = master mix.

and instructor notes for evaluating student learning can be found in Appendix 5. Lab reports should contain the following sections: title, purpose, materials and methods, results, discussion, reflections and opinions, and references. It should be noted that the grading rubric used for this activity (Appendix 5) is a general lab report rubric for introductory students and contains a hypothesis section. Since students do not formulate a hypothesis to solve this crime, students should be reminded to not complete this section and should instead be given five free points on their laboratory reports. Students should not be penalized if they include a hypothesis in their reports. As noted above, students should also include answers to the discussion questions in the discussion section of their laboratory reports. These are graded based on completion and accuracy. Each answer is worth two points, with one point deducted for inaccurate answers and two points deducted for omitted responses. Finally, the reflections and opinions section in the report is an opportunity for students to reflect on the activity. Students should be encouraged to reflect openly without fear of being penalized for negative opinions.

Student learning outcomes can be further assessed through the completion of pre- and post-activity quizzes. Quiz questions and an answer key are available in Appendix 6. In addition to questions assessing the student learning outcomes, the pre- and post-quizzes include two questions about DNA charge and directionality. These are important foundational concepts that students need to grasp in order to understand how agarose gel electrophoresis and PCR work, respectively.

## Sample data

Refer to Appendix 4 for a description of the *in silico* PCR and BLAST results from part 1 of the laboratory activity. Both the dog and human PCR primers amplify genomic fragments of the cardiac actin gene (4). Representative student agarose gel electrophoresis results from a PCR depicting Lady (human) as the culprit can be found in Figure 2. As expected, the Kona (dog) control with the dog PCR master mix yielded a band of 275 bp and the Lady (human) control with the human PCR master mix yielded a band of 397 bp. This gel reveals Lady (human) as the culprit since there is an approximately 400 bp band in the crime scene sample with the human master mix, and no band in the crime scene sample with the dog master mix.

A representative lab report from a student who received a high score can be found in Appendix 5. Note that the student included a hypothesis section and was not penalized for this inclusion.

## Safety issues

This exercise is designed for a Biosafety Level 1 (BSL-1) laboratory (6); standard laboratory personal protective equipment (PPE) should be worn during parts 2 to 4. Additional safety precautions include:

1. Reminding students to not swirl hot agarose solution near their faces, to prevent burns
2. Providing appropriate UV protection while imaging agarose gel electrophoresis results
3. Proper handling of GelRed, a DNA intercalating dye for UV detection of DNA during agarose gel electrophoresis

Students should receive basic laboratory safety training prior to the commencement of this activity. Refer to the Instructor Materials (Appendix 2) for additional safety information.

## DISCUSSION

### Field testing

This laboratory exercise was developed at North Carolina State University (NCSU) for an introductory biotechnology course for first-year students. The course was a general education course that counted towards either the university's natural science or interdisciplinary perspectives requirements (12). Student learning outcomes were assessed during the fall 2014 (16 students) and spring 2015 (7 students) semesters. The majority of the students in both courses were non–life science majors (Table 1).

The course that this activity was field tested in met twice a week, with each session being a 4-hour combined lecture and laboratory session. All three of the lectures were disseminated before starting the laboratory component. The laboratory component followed the flow outlined in Figure 1, with other course material not relevant to this activity performed during the remaining class time.

### Evidence of student learning

Instructor observations and lab report results revealed that all students successfully predicted PCR amplicon size and sequence using *in silico* PCR and determined, using BLAST, that genomic portions of the dog or human cardiac actin gene would be amplified (student learning outcome 2). Furthermore, all students successfully determined the concentration and purity of the DNA samples and diluted samples for PCR (student learning outcome 3). Most PCRs were successful and all students were able to appropriately analyze agarose gel electrophoresis results to determine who committed the crime (student learning outcome 4). Finally, all students were able to use BLAST to analyze Sanger sequencing results (student learning outcome 5). The class averages on the lab report (±standard error of the mean; SEM) were 84.2% (±2.6%) for fall 2014 and 86.2% (±4.5%) for spring 2015, demonstrating students' attainment of the activity learning outcomes. Students mostly lost points for submitting lab reports with formatting errors, such as unlabeled figures and/or missing figure legends. Students also lost points for including only figures and tables, with no narrative description of their experimental findings in the results section. This was not surprising given that many students were writing lab reports for the first time and these are common mistakes of novice students. Feedback from these lab reports provided students an opportunity to improve their technical writing skills for current and future science courses. Refer to Appendix 5 for a representative lab report from a high-achieving student. As mentioned previously, students should respond to discussion questions in the discussion section of the lab report. Refer to Appendix 2 (Instructor Materials) for discussion questions and representative student responses.

Prior to the introductory lectures and laboratory exercise, students completed a multiple-choice quiz (Appendix 6). Students completed the same quiz at the conclusion of the laboratory. These quizzes assessed all student learning outcomes and contained two additional questions on DNA charge and directionality, which are foundational concepts needed for an understanding of agarose gel electrophoresis and PCR, respectively. Analysis of the quiz data using a Wilcoxon signed-rank test revealed significantly improved grade distributions on the posttest compared to the pretest (Fig. 3), demonstrating that the student learning outcomes of the activity were achieved. As shown in Table 2, questions associated with student learning outcomes 1, 2, 4, and 5, as well as the questions on DNA charge and directionality, had the strongest gains. Low gains were observed for student learning outcome 3. For the purity question, students scored high on the pre-quiz due either to previous knowledge or a "lucky guess," which resulted in the low gains. For the DNA concentration question, students did worse on the post-quiz than the pre-quiz. This may be the result of students using a DNA spectrophotometer that automatically calculated DNA concentrations instead of having to calculate based on A260 values.

Using a five-point Likert-type scale, where 1 denoted strongly disagree and 5 denoted strongly agree, students responded to an anonymous post-activity survey (Appendix

TABLE 1.
Student majors in the fall 2014 and spring 2015 semesters.

| Major | Fall 2014 | Spring 2015 |
|---|---|---|
| Life science[a] | 4 | 2 |
| Non–life science | 12 | 5 |

[a] Life science majors include biology, biochemistry, biomedical engineering, genetics, and human biology.

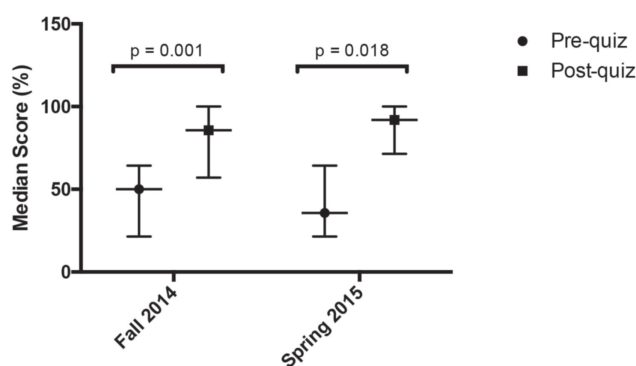


FIGURE 3. Pre- and post-quiz results from the fall 2014 and spring 2015 semesters. Median scores (with upper and lower limits) are displayed, with 16 students completing the pre- and post-quizzes in fall 2014 and seven students completing the quizzes in spring 2015. Data was analyzed with a Wilcoxon signed-rank test (SPSS Statistics, version 22), with *p*-values displayed.

TABLE 2.
Pre- and post-quiz responses reveal gains in student learning outcomes (SLOs).

| | | Number of Correct Responses (*n* = 23) | |
|---|---|---|---|
| **SLO** | **Question Topic** | **Pre-quiz** | **Post-quiz** |
| 1 | *Taq* polymerase | 13 | 22 |
| | Annealing of PCR primer | 8 | 15 |
| | PCR thermocycling conditions | 9 | 20 |
| | Exponential nature of PCR | 21 | 23 |
| | Sanger sequencing primers | 10 | 21 |
| | Dideoxynucleotide triphosphate (ddNTP) | 7 | 20 |
| 2 | *in silico* PCR | 12 | 20 |
| 3 | DNA purity | 16 | 17 |
| | DNA concentration | 12 | 11 |
| | Dilutions | 9 | 18 |
| 1, 4 | Analyze agarose gel electrophoresis results | 5 | 19 |
| 5 | Sequencing analysis with BLAST | 12 | 17 |
| N/A | DNA charge | 5 | 20 |
| N/A | DNA directionality | 6 | 22 |

SLO = student learning outcome; PCR = polymerase chain reaction; DNA = deoxyribonucleic acid; BLAST = basic local alignment search tool; N/A = not applicable.

TABLE 3.
Results of post-activity student perceived learning gains assessment.

| SLO | Skill or Conceptual Knowledge Gained | Average Ranking (SEM) |
|---|---|---|
| 1 | An understanding of DNA agarose gel electrophoresis | 4.19 (0.21) |
| | An understanding of PCR | 3.86 (0.21) |
| | An understanding of DNA sequencing | 3.86 (0.19) |
| 2 | Use bioinformatics tools to predict genes to be amplified during PCR | 4.05 (0.13) |
| 3 | Calculate the concentration of DNA | 3.95 (0.13) |
| | Determine the purity of DNA | 4.05 (0.16) |
| 4 | Analyze PCR results | 4.05 (0.18) |
| 5 | Use bioinformatics tools to analyze sequencing results | 4.19 (0.11) |

SLO = student learning outcome; SEM = standard error of the mean; DNA = deoxyribonucleic acid; PCR = polymerase chain reaction. Data are representative of 21 students, 16 from fall 2014 and 5 from spring 2016.

6) on their perceived gains in skills and conceptual abilities associated with the activity's learning outcomes. As shown in Table 3, student responses revealed perceived gains in each of the outcomes. The highest perceived gains were associated with determining the purity of DNA, understanding the purpose of agarose gel electrophoresis, analyzing PCR results, using bioinformatics tools to analyze sequencing results and using bioinformatics tools to predict genes to be amplified during PCR.

### Possible modifications

This activity is purposefully introductory; it is designed for novice students. However, modifications could be made to further augment discipline-specific critical thinking. One potential modification is to ask students to design their own experiments to solve the hypothetical crime after completion of the laboratory component of the activity. Students could design novel primers to the cardiac actin gene in dogs and humans or be directed to DNA and protein sequences that differ between the two species. A candidate protein would be myristoylated alanine-rich C-kinase substrate (MARCKS) (9), where the dog protein sequence shares 66.3% identity to human protein. Students could use BLAST to compare the dog and human sequences and describe the relationship by evaluating the E-values. Using the GenBank and RefSeq databases, students could also design their own primers, verifying primer specificity and that a single amplicon would be generated with each primer pair using *in silico* PCR and BLAST. Students could then design a PCR protocol, to include calculation of expected annealing temperatures, and submit a write-up of expected results if either Kona or Lady was the culprit. If time and resources allow, students could execute their experiments and compare results to the laboratory experiment described here. For this modification, a more detailed explanation of PCR, including primer design and development of a thermocycling procedure, would need to be provided. Additionally, the bioinformatics resources would need to be expanded to include detailed use of GenBank and RefSeq databases and how to locate intron and exon sequences. Refer to Appendix 7 for a description of other possible modifications to this activity.

## SUPPLEMENTAL MATERIALS

Appendix 1: Lecture materials
Appendix 2: Instructor materials
Appendix 3: Student handout
Appendix 4: Sequencing results
Appendix 5: Lab report guidelines, rubric, and sample lab report
Appendix 6: Pre- and post-quiz with answer key
Appendix 7: Other possible activity modifications

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