Article

Aligning Goals, Assessments, and Activities: An Approach to Teaching PCR and Gel Electrophoresis

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Polymerase chain reaction (PCR) and gel electrophoresis have become common techniques used in undergraduate molecular and cell biology labs. Although students enjoy learning these techniques, they often cannot fully comprehend and analyze the outcomes of their experiments because of a disconnect between concepts taught in lecture and experiments done in lab. Here we report the development and implementation of novel exercises that integrate the biological concepts of DNA structure and replication with the techniques of PCR and gel electrophoresis. Learning goals were defined based on concepts taught throughout the cell biology lab course and learning objectives specific to the PCR and gel electrophoresis lab. Exercises developed to promote critical thinking and target the underlying concepts of PCR, primer design, gel analysis, and troubleshooting were incorporated into an existing lab unit based on the detection of genetically modified organisms. Evaluative assessments for each exercise were aligned with the learning goals and used to measure student learning achievements. Our analysis found that the exercises were effective in enhancing student understanding of these concepts as shown by student performance across all learning goals. The new materials were particularly helpful in acquiring relevant knowledge, fostering critical-thinking skills, and uncovering prevalent misconceptions.

INTRODUCTION

Molecular techniques are commonly taught in undergraduate biology lectures and labs as a way to demonstrate how a scientific hypothesis is tested and to help students understand the primary scientific literature taught in typical lecture courses. However, the complexity of molecular techniques often makes it difficult for students to connect the technique to the basic biological principles. Furthermore, molecular techniques are often taught using a list of instructions without a direct tie to the biological concepts that are necessary for a comprehensive understanding. For these reasons, students often struggle to fully analyze data completely, draw appropriate conclusions, or troubleshoot problems related to the technique.

Previous studies have shown that the combination of "hands-on and minds-on components" of a lab help increase student learning of biological concepts (Halme *et al.,* 2006). Research-oriented labs promote the application of critical-thinking skills to real-world scientific problems (Smith *et al.,* 2005; Handelsman *et al.,* 2007) and the development of scientific reasoning by allowing students to investigate a problem and make conclusions about their results. By learning the biological principles underlying the techniques, students develop higher-order critical-thinking skills and can correct misconceptions in a way that promotes knowledge retention. Critical-thinking skills are promoted by analysis, synthesis, and evaluation questions as defined by Bloom's Taxonomy (Bloom and Krathwohl, 1956; Yuretich, 2003). These skills require students to integrate scientific knowledge and reasoning in order to make sense of new concepts or techniques and extend their knowledge to new contexts.

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Polymerase chain reaction (PCR) and gel electrophoresis, lab techniques, which are now commonly taught at the undergraduate level, offer a platform to combine wet labs and inquiry-based exercises that target the biological concepts. In doing so, the students have the opportunity to learn molecular techniques in the context of a real-world situation using a diversity of learning methods. We designed several research-oriented exercises that incorporate biological principles with data analysis in a variety of contexts to be used as additions to a wet lab unit. Specifically, the wet lab and teaching materials we describe here target the biological concepts of PCR and gel electrophoresis in the context of detecting genetically modified organisms (GMOs). The wet lab entitled: "What kind of genes are in your Fritos?: Detecting Genetic Modification in Food by Polymerase Chain Reaction" (in the unpublished Biocore 304 Cell Biology lab manual by J.B. and D. Bradner) is taught over a 2-wk period during which students isolate DNA from common food products containing soy and corn and use *CaMV35S* promoter-specific primers, PCR, and gel electrophoresis to determine the presence or absence of genetically modified ingredients in the products. The *CaMV35S* promoter is a constitutive promoter often used to express transgenes in GMOs and is therefore an acceptable marker for the presence of GMOs. The teachable unit (i.e., the instructional materials that include learning goals, assessments, and activities; Handelsman *et al.,* 2007) that we designed to complement this lab encompasses four hands-on exercises that expose students to typical challenges associated with performing and analyzing PCR results in the form of DNA gels. Ultimately, the hands-on lab components and the research-oriented paper exercises help students to evaluate data critically and draw conclusions for a presentation at the conclusion of the unit.

Previously, the goals of the wet lab were largely directed toward understanding the technology behind GMOs and discussing the pros and cons of such technology with little emphasis on the concepts underlying PCR and gel electrophoresis. The instructors noted then that the students struggled to discuss the processes of PCR, gel electrophoresis, and gene transfer at the molecular level. This corresponded to an inability to explain unexpected results or troubleshoot problems during the experiment. For example, students were typically unable to explain missing or unexpected bands on their DNA gels. To enhance their understanding of these molecular topics, we created a series of research-oriented exercises that targeted the theoretical concepts of PCR, gel electrophoresis, and primer design and allowed students to practice troubleshooting these lab techniques and unexpected results (Allen and Tanner, 2003).

There are many challenges for instructors teaching molecular techniques for the first time, including identifying and actively correcting misconceptions and devising assessments to measure student understanding of the learning goals. The assessments we designed allowed us to evaluate student learning of the established goals and the effectiveness of the exercises in teaching the concepts. Through student comments and assessments, we identified potential issues that led to misunderstandings of the exercises or concepts, and we subsequently made revisions to clarify the misunderstandings. These types of data-driven decisions improve materials and teaching methods, resulting in an increase in student understanding.

We designed the teachable unit using scientific teaching as the framework for research-oriented learning and the promotion of critical-thinking skills (Handelsman *et al.,* 2004, 2007). We also followed the backward design process of Wiggins and McTighe (1998), in which learning objectives are systematically aligned with the learning assessments and activities (Handelsman *et al.,* 2007). We used the objectives of the wet lab as a framework to establish the learning goals for the teachable unit and created a diverse group of research-oriented exercises to help students achieve these goals. This article describes multiple methods of assessment and evaluation that were used to gauge the level of student performance, referred to here as learning gains or losses (Huba and Freed, 2000; Handelsman *et al.,* 2007).

In this article, we present our results that demonstrate student learning gains on the concepts of PCR and gel electrophoresis, which correspond to the learning goals established at the onset of designing the teachable unit. Students were actively engaged in discussing and completing the new materials, which allowed for the identification and clarification of misconceptions by both instructors and students. This was possible because we aligned learning goals, curricular activities, and student performance and perception assessments.

TEACHABLE UNIT DESIGN

Classroom Context

The teachable unit was implemented in an honors, introductory, undergraduate, cell biology lab course. The course is paired with a lecture course, both of which are nested in a four-semester core biology curriculum (University of Wisconsin–Madison Biocore; http://polyglot.lss.wisc.edu/biocore/index.html) covering ecology, evolution, genetics, cell biology, and organismal physiology (Batzli, 2005). Students in this course are highly motivated sophomores and juniors, many who will continue their education in postbaccalaureate programs. Most had little experience with molecular biology techniques. The 2-wk GMO lab unit consisted of three 50-min discussion periods and two 3-h lab periods during which students carried out DNA isolation, PCR, and gel electrophoresis, using time between benchwork to complete the paper exercises (Figure 1).

Learning Goals

The first step in backward design is defining the broad and specific student learning goals for the teachable unit. As defined here, broad learning goals address general science skills, such as making careful observations, designing quality materials for experiments, and troubleshooting experiments. All of these skills ultimately help students analyze and present scientific data through evaluation of experimental design, which includes recognition of assumptions and the significance of controls (Table 1). These goals represent the true nature of scientific inquiry and the students encounter them repeatedly throughout the Biocore curriculum. The broad goals provide a framework for the specific goals that we measured during this unit. These specific goals address

topics targeted to the wet lab and teachable unit such as understanding PCR concepts, interpreting DNA gels, and designing primers (Table 1). Both broad and specific goals cover a range of competencies from simple knowledge to higher-order synthesis and evaluation questions, as defined by Bloom's Taxonomy (Bloom and Krathwohl, 1956).

We used both broad and specific goals to guide development of five exercises: PCR Cycle Sketch, PCR Exercise, Primer Design Exercise, Gel Analysis Exercise, and a final presentation, that comprise the teaching unit (Figure 1). Brief activities, including instructor mini-lectures, student diagramming, problem-solving exercises, computer work, and presentations, were developed to encompass the many learning styles of a diverse student population (Tanner and Allen, 2004). The exercises were designed to complement a previously established lab unit; however, each exercise can be used individually or as part of a wet lab to address specific goals. For each exercise, we developed multiple assessments to measure student performance, perceptions,

and learning gains (Huba and Freed, 2000). As shown in Figure 1, we systematically aligned the exercises and assessments with the broad and specific goals to provide a measure of achievement for each. This ensured that all goals were assessed at least once during the unit and provided a framework for assessment throughout the unit.

PCR Cycle Sketch

The goal of the PCR Cycle Sketch (Supplemental Material A) was to help students visualize each step of PCR and understand primer annealing and directionality of DNA replication. It contains a series of questions about PCR and was incorporated into an existing prelab assignment (not included). Students were expected to complete this exercise before coming to class. The exercise consisted of the following activities: 1) watching short virtual representations of the steps of PCR (Dolan Learning Center, 2006a; Sumanas, 2006) and gel electrophoresis (Dolan Learning Center, 2006b); 2)

Figure 1. Scientific teaching and alignment of the teachable unit. Teachable unit overview. The flow chart provides a time line of the GMO lab (gray) and how each newly developed exercise (black) was implemented. Each exercise was aligned with the broad and specific goals and evaluated for a level of understanding with several types of assessments. An array of teaching methods was utilized to accommodate diverse learning styles. Specific Goals: 1, drawing the steps of PCR; 2, troubleshooting inferences; 3, gel electrophoresis; 4, using a DNA ladder; 5, positive and negative controls; 6, designing primers; and 7, GMO case-based presentations.
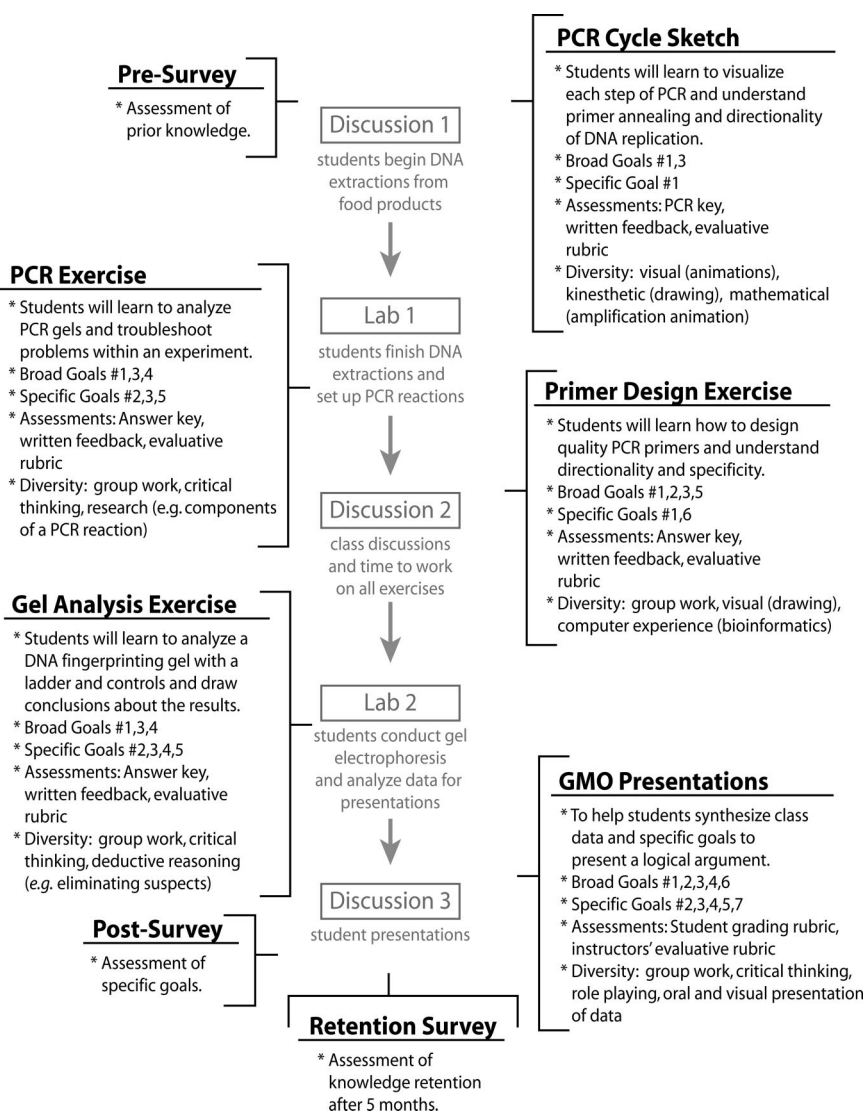
hand-drawing the first three cycles of PCR, indicating the proper intermediate and final products and appropriate directionality of the primers; and 3) answering several questions about the ratio of intermediate and target DNA products and how that relates to DNA amplification during PCR. We took time in the subsequent class to discuss with students several misconceptions about PCR that were revealed by their responses to the pre-lab assignment (see a summary of these and other misconceptions in Table 2).

PCR Exercise, Primer Design Exercise, and Gel Analysis Exercise

Worksheet exercises (Supplemental Materials B–D) were designed to help students familiarize themselves with primer design and provided several examples of DNA gels in different contexts for students to analyze. Students worked in groups of two to four, as time allowed in the lab, to complete

these exercises. The worksheets were designed to give students 1) exposure to multiple problems that may occur in the lab while carrying out PCR and gel electrophoresis and experience troubleshooting these problems, 2) instructions for and experience with designing primers for experimentation, and 3) experience with PCR data analysis in the context of DNA fingerprinting. Students analyzed results from an array of gels, predicted the results of an experiment, and drew conclusions about these results. These exercises were completed in class before students analyzed their own results, to prepare them to be more critical of the conclusions subsequently made from their own data.

Final GMO Case-based Presentations

After the completion of the GMO lab and all associated exercises, students were challenged to develop a logical, data-driven argument to support their assigned position in a mock trial regarding the presence or absence of GMOs in a particular food product to be presented during the final discussion period (Supplemental Material E). Students, in groups of four, analyzed PCR gel images from the compiled class data and determined which results provided evidence for their case. They presented their positions in formal presentations for the class and were expected to integrate their data analysis to support their position and answer questions. All student presentations were videotaped for later evaluation.

Assessments

Formative and summative assessments were used to evaluate student performance and learning gains (Handelsman *et al.*, 2007). Pre- and postsurveys and retention surveys (see http://scientificteaching.wisc.edu/materials) were created to gauge student perceptions, learning gains, and knowledge retention. Questions focused on student perceptions of the new materials, understanding of DNA amplification by PCR, primer design, and positive and negative controls in experimental design. Students completed an online presurvey before the start of the unit and a postsurvey after the presentations (Figure 1). Student responses to presurveys were used to establish a baseline of prior knowledge, whereas postsurvey responses were used to assess specific goals and as a comparison for knowledge retention. We also assessed student knowledge retention via a paper survey 5 mo after completion of the unit. This survey was conducted in the next class of the Biocore series, which aided in student participation. All surveys were completed in class to ensure uniformity in testing conditions. Data presented are averages of the responses from 97 participants (exempt from review by UW-Madison Institutional Review Board [IRB] protocol no. 2003-5221).

Students were formally graded on the PCR Cycle Sketch, Primer Design Exercise, and Final GMO Case-based Presentations. A rubric was specifically designed to assess student performance on the presentations (available online). Informal comments were provided by instructors for the PCR and Gel Analysis Exercises. In addition, students frequently received verbal feedback from instructors as they progressed through the exercises and the unit as a whole.

Table 1. Student learning goals

Broad learning goals
1. Students will understand how scientists ask questions.
2. Students will be able to select and/or design quality reagents for experiments.
3. Students will understand the importance of each step of a reaction or experiment.
4. Students will be able to make conclusions about data and connect technical results with biological and societal relevance.
5. Students will be able to use bioinformatics tools to gather information to aid in experimental design.
6. Students will be able to present scientific information in formal and semi-formal environments to their professors and peers.

Specific learning goals
1. Students will be able to draw each step of PCR accurately and label the temperature of each step, the directionality of the primers, the proper intermediate products, and the final products.
2. Students will be able to make troubleshooting inferences through reflection on the concepts of PCR and gel electrophoresis to determine possible problem areas.
3. Students will be able to explain how DNA molecules move through an agarose gel matrix, that the molecules are separated by size and weight, and why DNA moves toward the positive pole.
4. Students will be able to determine the size of a band in an agarose gel by using a DNA ladder.
5. Students will be able to interpret positive and negative controls correctly.
6. Students will be able to design quality PCR primers using bioinformatics databases.
7. Students will be able to analyze data and use the results to support a position for the presence or absence of GMOs in food products for a case-based presentation.

Broad and specific learning goals were determined before designing the teachable unit. Broad goals entail general concepts and techniques that encompass the entire wet lab and teachable unit and/or are applicable to the broader learning goals of the cell biology lab. Conversely, specific learning goals are concepts or techniques to be learned and measured in the GMO lab and teachable unit.

Evaluative rubrics were designed to assess all exercises and presentations (available online) for the purpose of determining the effectiveness of the new materials in reaching the learning goals (Huba and Freed, 2000). These separate evaluations did not have any impact on the students' grades and were only used as a research tool by the authors. Concepts evaluated by the rubrics were determined from the broad and specific goals and were aligned with the exercises (Figure 1). The videotaped student presentations were evaluated after completion of the unit for appropriate use of PCR concepts, correct analysis of data, and development of a logical scientific argument to support a group's position. To increase the validity of the evaluations and reduce bias, each group presentation was evaluated by the authors and three other scientists not involved in this project or related to

Biocore, but familiar with the techniques and theories being evaluated. Final group scores were the average of all evaluator scores.

RESULTS

Presurvey Responses

The presurvey asked students to report their level of understanding about directionality, primers, PCR, and positive and negative controls. Approximately 65–70% of students reported they were unsure of these topics, whereas 30–35% of students felt they understood the topic. The topic of directionality was more specifically evaluated on the presurvey when students were asked to write the reverse comple-

Table 2. Summary of common misconceptions and how they were addressed

Common misconceptions[a]	Addressing the misconception
Specific Goal 1: drawing the steps of PCR	
Both the forward and reverse primers bind to the sense strand of the DNA template. (PCR Cycle Sketch)	Students drew both strands of DNA and located the site of primer annealing, indicating directionality on both the template DNA and primers
Direction of DNA replication can be $5' \to 3'$ or $3' \to 5'$. (PCR Cycle Sketch)	Students identified with arrows the direction in which DNA replication should occur during PCR.
PCR primers cut or isolate but do not amplify DNA. (PCR Cycle Sketch)	Students watched a PCR animation detailing amplification and had a class discussion about the differences between primers and restriction enzymes.
All DNA is amplified in a PCR reaction. (PCR Cycle Sketch)	Students answered questions about amplification of target DNA vs. template or intermediate DNA.
Specific Goal 2: troubleshooting inferences	
Composition of each reagent in a reaction is not important. (PCR Exercise)	Students completed a worksheet detailing the function of each reagent in PCR and gel electrophoresis and had to troubleshoot problems due to incorrect reagents.
Specific Goal 3: gel electrophoresis	
Can determine the amount of DNA on an agarose gel. (Gel Analysis Exercise)	Students participated in group discussions about what can be inferred from an agarose gel.
Template DNA disappears during PCR so we cannot see it on the gel. (PCR Cycle Sketch, PCR Exercise)	Students demonstrated DNA amplification by drawing PCR steps on the board and answered questions on exercises about intermediate bands.
Specific Goal 4: using a DNA ladder	
Larger pieces of DNA run faster than smaller pieces of DNA. (Gel Analysis Exercise)	Students worked together to form a hypothesis about what a gel should look like and then drew the gel on the board.
Specific Goal 5: positive and negative controls	
There is no difference between the "No DNA" control and the "Negative DNA" control. (Gel Analysis Exercise)	Students completed an exercise that required analysis and explanation of all controls and how they influence conclusions.
Specific Goal 6: designing primers	
Both the forward and reverse primers bind to the sense strand of the DNA template. (Primer Design Exercise)	Students designed forward and reverse primers by drawing both strands of DNA with appropriate base complementarity and indicated primer annealing.
BLAST results should not include the gene of interest. (Primer Design Exercise)	Students answered questions and had one-on-one discussions about the importance of the best BLAST matches.
BLAST results within the organism of interest are the only important matches. (Primer Design Exercise)	Students participated in a group discussion about cross-reactivity of primers and the importance of BLAST matches in multiple organisms.
Specific Goal 7: GMO case-based presentations	
Only need to analyze and present the data that supports my position. (GMO presentations)	Students participated in a class discussion about the importance of considering *all* data when drawing conclusions about an experiment and the error in "spinning" data.

The table delineates the most prevalent misconceptions from the GMO lab. Misconceptions are separated by specific learning goals in the left column and paired with the corresponding active learning technique used to address the misconception in the right column. The exercises that helped to identify each misconception are listed in parentheses.
[a] Misconceptions are stated as student misinterpretations.

ment of a DNA sequence. Although 12% of students responded correctly, 88% of students could not successfully write the reverse complement. We used the data from the presurvey as a baseline of students' prior knowledge.

Learning Goals

Exercises were designed to target the learning goals established for the unit (Table 1 and Figure 1). Each exercise was evaluated with its own rubric containing three to four questions, each aligned with appropriate broad and specific goals, and scored on a trilevel scale, with three describing the highest level of comprehension (available online). Data are presented as the percentage of students who achieved a level one, two, or three of understanding. Datum gathered for each specific goal across multiple exercises was averaged to give an overview of how well students achieved the learning goals (Figure 2). For example, specific goal two (Table 1) was assessed by one question from the PCR Exercise and two questions from the Gel Analysis Exercise. All three questions examined how well students could make troubleshooting inferences. Seventy percent of students attained a level three, 25% a level two, and 5% a level one for this learning goal. For specific goals one through six, the majority of students attained a level three of understanding, thereby demonstrating comprehension of the steps of PCR and gel electrophoresis, interpretation of PCR results using controls and DNA size standards, design of quality primers, and troubleshooting experimental results (Figure 2A).

Assessment of specific goal seven was based on final group presentations and was scored on a separate five-level rubric, levels zero through four (available online; Figure 2B). Students were asked to synthesize the information contained in all of the other exercises to formulate a logical argument based on class data for or against the presence of GMOs in a particular granola bar. The data were used as evidence for their argument in a mock trial regarding false advertisement and GMOs. The majority of students, 65%, obtained a level three or four, indicating a clear, logical, and complete interpretation of the results (Figure 2B).

An Example Assessment: Gel Analysis Exercise

Figure 3 highlights data from the Gel Analysis Exercise as an example of how each exercise was evaluated. Similar datum from each of the five exercises was compiled for each specific learning goal to create Figure 2. To assess individual exercises, an evaluative rubric was designed with specific questions that aligned with the broad and specific learning goals. The Gel Analysis Exercise (Supplemental Material D) asked students to identify suspects based on PCR fingerprinting results. Students used a DNA ladder and positive and negative controls to identify cross-reacting bands and appropriately eliminate suspects. Four questions were used to evaluate student learning gains on the exercise, which covered specific goals two, three, four, and five (Table 1 and Figure 1).

The first question asked how well the students could identify cross-reacting bands using positive and negative controls. Eighty percent of students correctly eliminated suspects (level three), whereas 19% could identify appropriate suspects to eliminate but misinterpreted the negative water control, which led them to errantly dismiss one suspect (level two). Question two assessed how well students were able to design the next logical experiment, which in this case was to retest a suspect because of lack of a cross-reacting band. Level three required students to identify which suspect to test and the appropriate controls to use (water and DNA standards that contain or lack the gene fragment). Sixty-three percent of students completed this successfully, whereas 31% could identify the suspect but did not include the appropriate controls (level two). Question three evaluated how well each student could interpret gel results using a DNA ladder. Ninety-six percent of students could determine the appropriate size of the bands based on the ladder, but only half of those students could state how size influenced their decision to eliminate suspects. The last question assessed student understanding of positive and negative controls and how they influenced the student's conclusions about suspects. The majority of students, 79%, were able to identify positive and negative controls (levels two and three); however, only 20% could convey how the
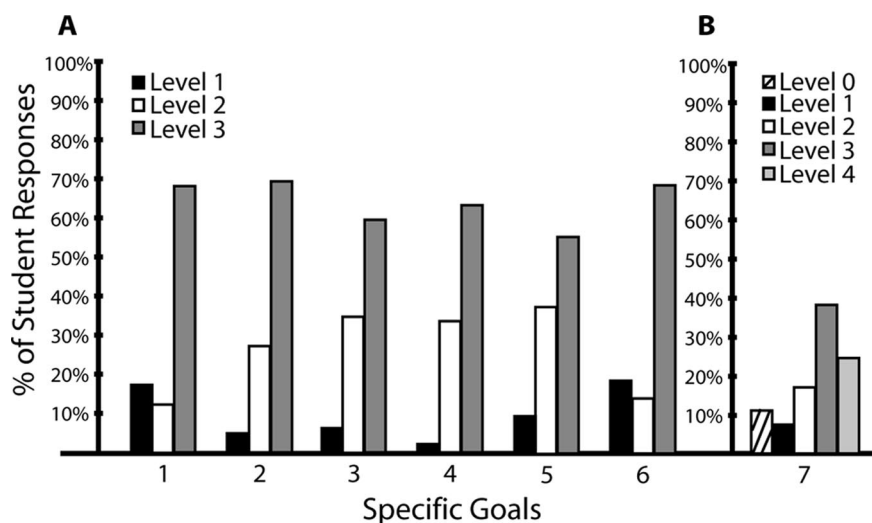

Figure 2. Student achievements on specific goals. Over the course of the teachable unit, students completed four exercises and a final project all designed to meet seven specific goals (Table 1). All specific goals corresponded to at least two independent questions on one or several exercises. Student understanding for each question was ranked according to two rubrics. Exercises were scored on a three-level scale (A), and the final project was scored on a five-level scale (B). All responses were compiled for each specific goal and are presented as a percentage of total responses (n = 97). Goals: 1, drawing the steps of PCR; 2, troubleshooting inferences; 3, gel electrophoresis; 4, using a DNA ladder; 5, positive and negative controls; 6, designing primers; and 7, GMO case-based presentations.

controls affected their decisions (level three). For example, some students could recognize a negative control but could not explain the importance of the presence of a cross reacting band in that control, which can be used as an internal control for a successful reaction.

Knowledge Retention

Students were surveyed 5 mo after completion of the unit to assess retention of knowledge gained during the unit (Figure 4). Retention questions were directly paired with three rubric questions used to assess the exercises. Question one addressed amplification of a specific DNA sequence using PCR and was paired with the same rubric question from the PCR Exercise. During the GMO unit, 78% of students attained a level three of understanding and 18% attained a level two of understanding. Five months later, 47% percent of students achieved a level three, whereas 39% attained a level two. According to students' responses, those who achieved a level one or two understood how PCR amplified DNA but still had misconceptions about the specificity of primers.

The second retention question required students to design a reverse primer with proper orientation similar to the Primer Design Exercise. During the unit, a great majority of students, 85%, clearly understood how to design a reverse
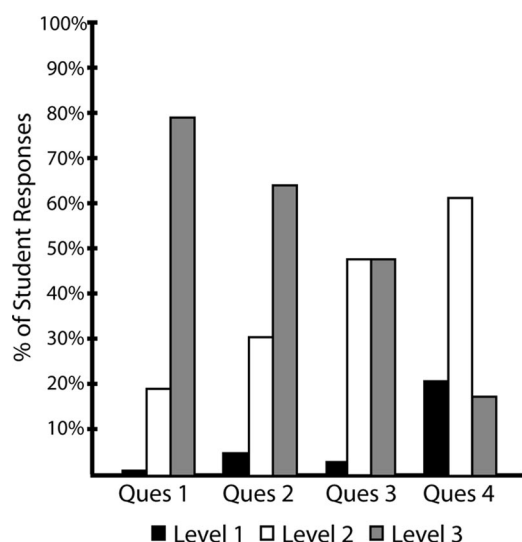
primer that was antiparallel to the sequence of interest and written in the 5′ to 3′ direction, in accordance with standard notation. Although 37% of students were able to design a correct reverse primer 5 mo later (level three), 42% mistakenly designed a forward primer, indicating confusion about the directionality of DNA replication or standard notation (level one). The remaining 21% of students designed a primer that was either a complementary sequence or had reverse directionality, but not both (level two).

Question three examined how well students understood positive and negative controls as first evaluated on the Gel Analysis Exercise. During the unit, 59 and 20% of students reached levels two and level three, respectively, indicating the majority of students could identify positive and negative controls and use them to interpret results from the lab. Sixty-six percent of students were able to define positive and negative controls correctly on the retention survey (level three). The percentage of students with a level one of understanding decreased from 21 to 5% after 5 mo.

Students' Perceptions of New Materials

On the postsurvey, students were asked about the usefulness of the materials and activities within the exercises in contributing to their understanding of various concepts related to PCR and gel electrophoresis. For each exercise, students selected whether the exercise was of no contribu-
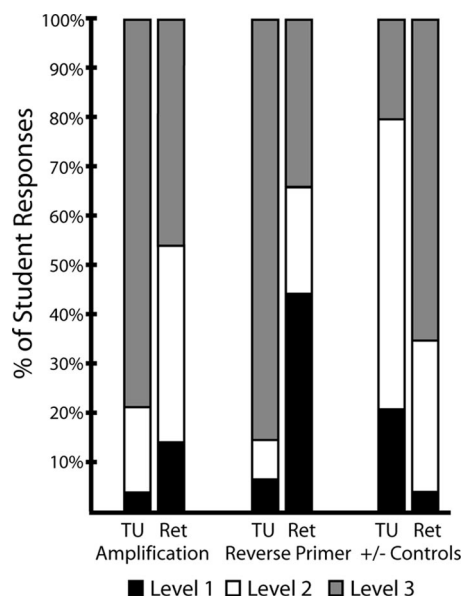

Figure 3. Levels of student understanding for a representative exercise. On the Gel Analysis Exercise, students were asked to analyze a gel to eliminate possible suspects from a crime. Students needed to determine the presence or absence of a band and the size of a band accurately to make their conclusions. Students' responses were evaluated for the following questions. Question 1, how well can the student analyze the results of the gel to identify cross-reacting bands and make appropriate conclusions? Question 2, how well does the student identify and design the next important experiment to test? Question 3, how well does the student understand how DNA migrates in a gel and how to use the ladder to determine fragment sizes? Question 4, how well does the student understand the importance of positive and negative controls? Student understanding was ranked on a three-level scale, with a level three describing the most complete answer, using a rubric. All responses are presented as a percentage of total responses (n = 97).

Figure 4. Knowledge retention. Students were tested for knowledge retention on three topics covered during the teachable unit (columns: TU) and 5 mo after completion of the teachable unit (columns: Ret). Question 1 (Amplification). How well can the student explain why PCR is an appropriate technique for amplification of DNA? Question 2 (Reverse Primer). How well does the student understand the proper orientation/design of the reverse primer? Question 3 (± Controls). How well does the student understand the importance of positive and negative controls? Student understanding was ranked on a three-level scale, with a level three describing the most complete answer, using a rubric. All responses are presented as a percentage of total responses (n = 97).
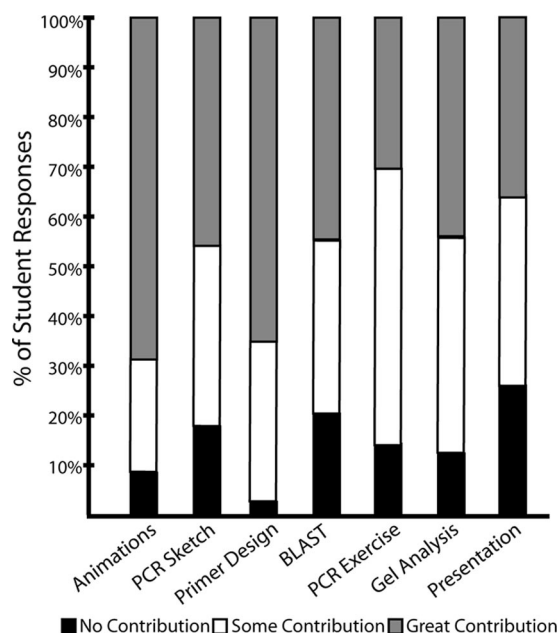
Figure 5. Students' perceptions of the utility of instructional materials. In a postsurvey, students were asked how useful the new materials were in understanding PCR and gel electrophoresis. Each column pertains to the exercises explained in the Teachable Unit Design section. ■, no contribution to student understanding; □, some contribution to understanding and/or clarified a misconception; and ▨, a great contribution to student understanding. All responses are presented as a percentage of total responses (n = 97).

tion, some contribution and/or clarified a misconception, or a great contribution to their understanding (Figure 5). A large majority of students responded that all exercises contributed in some way to their understanding (>80% for each exercise). Students felt the animations and primer design exercise were most helpful, with greater than 60% reporting that these exercises were of great help.

DISCUSSION

The premise of scientific teaching guided our systematic alignment of learning goals, activities, and assessments, which helped us identify many misconceptions (Figure 1). By using this process we observed an increase in student performance and meaningful learning gains for all specific goals (Figure 2). This type of authentic assessment of new instructional materials allowed us to measure student learning and knowledge retention of PCR and gel electrophoresis in a rigorous way as a model of scientific teaching.

Assessment of Student Performance

The seven specific goals (Table 1) were framed in light of the biological concepts necessary for understanding PCR and gel electrophoresis, thus allowing students to troubleshoot aberrant results better and accurately present data. Evaluative rubric questions, which address the specific learning goals, were designed for each exercise (available online). The alignment of all seven specific goals to multiple exercises and rubric ques-

tions allowed us to assess overall student performance as depicted in Figure 2. This alignment ensured that the learning goals were covered in several ways during the 2-wk lab unit (Figure 1). Repeated exposure to targeted concepts helped the majority of students achieve the highest level of understanding for specific goals one through six (Figure 2).

The Primer Design Exercise (Supplemental Material C) provided a means to assess specific goal six, including students' understanding of primer design, directionality, and specificity across multiple levels of understanding (Bloom and Krathwohl, 1956). Students were challenged to comprehend, analyze, and synthesize information leading to a broader distribution of understanding for this specific goal (Figure 2). The similarity of the data for specific goal six and the more extensive data collected for the Primer Design Exercise alone led us to conclude that Figure 2 is a reasonable presentation of overall learning gains.

The concepts covered on the exercises built on each other as the students progressed through the unit such that the final presentation required an in-depth understanding of all concepts pertinent to the specific goals. This is evident with specific goal seven (Figure 2) because students had to synthesize all available data to support their position in a succinct 5-min presentation. Many students were able to make a complete, logical, scientific-based argument. Those who did not make a complete argument tended to focus on one particular aspect such as primer quality and failed to completely analyze all pertinent data. This may be a result of misinterpretation of the assignment, time restrictions during presentations, or incomplete data analysis. For example, the original assignment required each group to present data in support of their position. Some groups interpreted this loosely and chose to present legal data as evidence so as not to overlap with other groups presenting scientific evidence. This presented additional challenges in assessing group presentations. The unintentional ambiguity of the assignment was clarified during the revision process with the final version provided as Supplemental Material E, which instructs students to present scientific evidence only. In the future, a more complete understanding of student knowledge could be further assessed by a short writing assignment focused on data analysis and interpretation.

Student understanding at the end of the unit notably differed from student responses on the presurvey. For example, on the presurvey 12% of the students could write the reverse complement of a given DNA sequence, whereas 88% of students could not successfully write the reverse complement. This incomplete understanding often led to misconceptions later in the unit (Table 2). In contrast, nearly 90% of students could write the reverse complement of a sequence on the Primer Design Exercise during the unit. Based on comparisons with these presurvey data and exercise evaluations, most students achieved the specific goals (Figures 2 and 3). Combined data gathered from student responses on the exercises correlated well with the ability of the entire class to discuss and apply the concepts of PCR and gel electrophoresis.

Knowledge Retention

Figure 4 depicts retention data for three topics, amplification of DNA during PCR, design of the reverse primer, and

positive and negative controls, 5 mo after completion of the unit. Though some knowledge was lost after 5 mo, retention scores were higher than the typical retention expectation of <50% of what they read, hear, or see (Felder, 1995). We predict that students who retained a level two or three of understanding (>50% for all three questions) would have the tools to apply the biological concept to a new context. Although the first two questions highlighted novel concepts, for which students had many misconceptions (see below), the third question about positive and negative controls is a concept students encountered many times throughout the semester in lab and lecture. Therefore, increased performance on this third concept was not surprising and a good indication that multiple exposures to a topic improves student knowledge retention.

Students' Perceptions of New Materials

Students had positive perceptions of new materials, as presented in Figure 5, which may have contributed in part to student performance. Throughout the unit students were very receptive to multiple learning media (Figure 1) such as animations (McClean *et al.*, 2005), diagramming, group work, class discussions, and role-playing (Tanner and Allen, 2004). Students responded particularly well to the Primer Design Exercise. This exercise had the advantage of grade-based motivation as part of the lab grade, and in addition, many students struggled with a similar assignment in lecture and therefore were more receptive to learning the concepts to aid their understanding. In contrast, students were less receptive to the BLAST search. During this exercise, they had to incorporate many new concepts such as combining primer specificity with new information on how to interpret BLAST results. This led to student questions that indicated misconceptions to the instructors. Although this exercise was useful in identifying misconceptions, the students were frustrated and perceived the materials as less helpful. We revised the Primer Design Instructions to help clarify the concept of specificity and its importance in primer design.

Using Research-oriented Learning to Identify and Clarify Misconceptions

While developing the exercises for this unit, we struggled to identify common misconceptions about PCR and gel electrophoresis. However, as we progressed through the unit many misconceptions were revealed by student responses and group discussions. We utilized many methods in an attempt to dispel these misconceptions and help students reconstruct their knowledge framework. The exercises were the first step for students to identify their own misconceptions, which were often addressed or targeted as they progressed through the exercises or through one-on-one, group, or whole-class discussions. Table 2 presents the most prevalent misconceptions grouped by specific goal and stated in terms of student misunderstanding. Each misconception is paired in the right column with the active-learning technique used to clarify the misunderstanding.

It was evident during the unit that the more common misconceptions arose multiple times, which allowed us to address the misunderstanding using diverse instructional and learning techniques. For example, students' misconceptions about primer annealing and directionality were evident during the PCR Cycle Sketch and often carried to the Primer Design Exercise. Students were first given the opportunity to draw the template DNA and show primer annealing during the PCR Cycle Sketch. Later, when designing primers, students worked in groups to determine primer annealing sites and how directionality influenced primer design. Both exercises, as well as class discussions, focused on specific goals one and six and dispelled the misconception that DNA can replicate in both the 5' to 3' direction and the 3' to 5' direction. This was evident when we compared the presurvey responses to responses on the Primer Design Exercise. Before the exercises, 81% of the students could write the complementary sequence or identify the correct direction of replication but could not combine these ideas to design an appropriate reverse primer for PCR. By the end of the unit, 85% of the students could synthesize both ideas.

Another pervasive misconception was that only target DNA is amplified during PCR, when in reality there is also a small amount of intermediate DNA present at the end of the reaction. Students' struggled with this concept during the PCR Cycle Sketch and the PCR Exercise as they answered questions about the ratio of template (including intermediate) DNA to target DNA and how that ratio can be formed into a hypothesis about how a DNA gel should look. As part of the exercises, students viewed animations about DNA amplification and participated in class discussions about the amplification of intermediate and target products during PCR, which aided in their understanding of amplification. The concept of amplification was important in reaching specific goals one and two, as students had to draw the final products of three cycles of PCR and make troubleshooting inferences about intermediate and target products. Figure 2 shows that students, in fact, did achieve high levels of understanding for specific goals one and two.

As instructors, we could not identify, let alone correct, all misconceptions the students held about PCR and gel electrophoresis; therefore, we have only presented the most prevalent misconceptions in Table 2. However, the alignment of the goals with exercises and assessments made it easier to identify misconceptions that were specific to the learning goals and correct those that were more fundamental to the biological concepts of PCR and gel electrophoresis. As misconceptions were written or vocalized, we had students use active-learning techniques, such as drawing to visualize microbiological events or forming hypotheses, in group or one-on-one discussions to help students correct their own misunderstandings and work to create a new knowledge framework about the concept. We found active-learning techniques to be incredibly helpful in both uncovering and correcting misconceptions and hope this list will aid other instructors in doing the same. We also view misconceptions uncovered here as starting points in developing additional targeted learning goals in future iterations of this unit.

Critical-thinking Skills

The teachable unit described here was developed as a research-oriented unit to enhance student understanding of biological concepts of PCR and gel electrophoresis and includes activities that require critical-thinking skills as identified by the National Science Education Standards (National

Research Council [NRC], 1996; Leonard and Penick, 2000; Handelsman *et al.,* 2007). For example, the PCR Exercise incorporates observing, identifying questions and problems, data analysis, making inferences from data, and formulating hypotheses. In addition to presenting their data, students were required to collect and organize data, display it such that inferences could be made, and communicate the results to peers and instructors. Students used critical-thinking skills to formulate a concise, logical argument using appropriate data to meet the goals of the presentation. All research-oriented exercises allowed for cooperative group work in which students were active investigators (Ahern-Rindell, 1998). The teachable unit additionally satisfies many of the collegiate goals of Bio2010 (NRC, 2003) by incorporating aspects of chemistry, computer science, and physics into a cell biology lab focused on teaching fundamental biological principles.

Transferability

All of the new materials were designed to enhance a previously established lab unit focused on detecting GMOs by PCR and gel electrophoresis. However, each exercise could be used independently as is or modified to be more applicable to a different context. For example, the PCR Cycle Sketch is a great addition to any lecture course to help students parse out the individual steps of PCR, or the context of the PCR Exercise could be modified to help students troubleshoot an experiment in a different organism. In fact, several of the exercises are being used in undergraduate mentoring programs and are used in Biocore for new labs associated with PCR and gel electrophoresis.

Final Comments

An important part of scientific teaching is the continuous revision of material based on data to improve student understanding. With this in mind, we redesigned several exercises in light of student misconceptions and to improve readability. The redesigned materials are those presented in Supplemental Materials A–E and can be accessed by the website listed below. We hope these exercises encourage others to create research-oriented exercises focusing on biological concepts to complement lab techniques. We have found that these exercises improved student understanding of molecular biology techniques and increased their ability to troubleshoot and analyze data. In addition, active learning is a practical way for instructors and students to identify misconceptions.

Accessing Materials

The final teachable unit exercises are included as appendices and can also be accessed with the surveys and all associated rubrics from http://scientificteaching.wisc.edu/materials.

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