

Article

Integrating PCR Theory and Bioinformatics into a Research-oriented Primer Design Exercise

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Polymerase chain reaction (PCR) is a conceptually difficult technique that embodies many fundamental biological processes. Traditionally, students have struggled to analyze PCR results due to an incomplete understanding of the biological concepts (theory) of DNA replication and strand complementarity. Here we describe the design of a novel research-oriented exercise that prepares students to design DNA primers for PCR. Our exercise design includes broad and specific learning goals and assessments of student performance and perceptions. We developed this interactive Primer Design Exercise using the principles of scientific teaching to enhance student understanding of the theory behind PCR and provide practice in designing PCR primers to amplify DNA. In the end, the students were more poised to troubleshoot problems that arose in real experiments using PCR. In addition, students had the opportunity to utilize several bioinformatics tools to gain an increased understanding of primer quality, directionality, and specificity. In the course of this study many misconceptions about DNA replication during PCR and the need for primer specificity were identified and addressed. Students were receptive to the new materials and the majority achieved the learning goals.

INTRODUCTION

Many undergraduates now have the opportunity to plan experiments as part of their biology course work. To take full advantage of this opportunity, students need to be grounded in the biological concepts (theory) of the procedures and understand the importance of the reagents used in these techniques. Labs teaching polymerase chain reaction (PCR) and gel electrophoresis provide the opportunity to introduce molecular biology techniques as well as an example of identifying appropriate reagents, in this case primers for PCR. In the Biocore Cell Biology lab at the University of Wisconsin–Madison (further described in Batzli, 2005, and <http://polyglot.lss.wisc.edu/biocore/>), these techniques are introduced in a research-oriented lab unit in which students investigate the presence of geneti-

cally modified organisms (GMOs) in common food products. In previous iterations of the lab, instructors found that students had difficulty analyzing the data from DNA gels and explaining aberrant results. For example, students often claimed contaminated reagents were the cause of unexpected bands in the gel. However, other sources of error, such as poor primer design or inappropriate annealing temperature, were not considered, possibly because of a lack of understanding of the biological basis of PCR. We hypothesized that learning the biology underlying PCR should improve students' ability to appropriately analyze data, troubleshoot experimental design, and connect the biological process of DNA replication to lab procedures, basic skills that can be readily applied to myriad other experiments. Therefore, we created a primer design instruction sheet and research-oriented exercise that would help students visualize where and how primers bind to cDNA and initiate DNA replication.

There is literature to support the introduction of primer design in undergraduate labs and workshops (Kim, 2000; and Shachack *et al.*, 2005); however, few measure student performance and learning gains during or after implementation of these new materials. Therefore, we purposefully

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aligned the learning goals with exercise questions to assess the effectiveness of our primer design materials in meeting the specific learning goals of the lab. We expected that our Primer Design Exercise would enhance student understanding and provide a basis for troubleshooting results from the lab. After doing this exercise, one student exclaimed, “Wow, I’ve been doing PCR in my lab for a year now and I never knew how it worked.” This statement highlighted the need to heighten student exposure to the theory behind lab experiments and the keys for a successful PCR reaction (such as understanding the necessary reagents and appropriate concentrations) instead of simply providing a protocol. The Primer Design Exercise also provided additional opportunities for students to gain experience with bioinformatics tools fulfilling an increasing need for the incorporation of bioinformatics into undergraduate biology classes (Honts, 2003; National Research Council, 2003).

This article highlights the process of designing and implementing an active-learning exercise about designing DNA primers for PCR using scientific teaching methods in order to achieve broad and specific learning goals (Handelsman *et al.*, 2004, 2007). During and after implementation, we assessed students’ perceptions and performance, which are presented below. We have evidence that this exercise helped students meet the specific learning goals of quality primer design, understanding primer directionality, and using bioinformatics to determine primer annealing specificity. Our experience indicates that these primer design materials can be used broadly to introduce students to the importance of quality biological reagents for DNA amplification and how to utilize bioinformatics databases.

METHODS OF INSTRUCTIONAL DESIGN

Study Participants

In spring 2006 there were a total of 97 study participants enrolled in the Biocore Cell Biology lab, who were divided among five lab sections of approximately 20 students each (approved by UW-Madison Institutional Review Board protocol no. 2003-5221). All of the students had previously or were concurrently taking the Cell Biology lecture course, which introduced the topics of DNA replication and PCR, with minimal coverage of primer design. Additionally, during the PCR teachable unit in lab, students learned basic principles of PCR and gel electrophoresis before working on the primer design materials.

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 use bioinformatics tools to gather information to aid in experimental design.
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 understand how to design quality PCR primers using bioinformatics databases.

Learning Goals

Instructional design was initiated by identifying student learning goals that were compatible with the pre-existing goals of the GMO unit and the cell biology lab in general. We outlined broad learning goals that include general skills and conceptual ideas that the students would repeatedly encounter throughout the course (Table 1). We also outlined two measurable, specific learning goals that were targeted to the Primer Design Exercise (Table 1).

Scientific Teaching

In designing the exercise we took into consideration the following pedagogical theories. Using the backward approach to instructional design (Wiggins and McTighe, 1998), which is critical in the implementation of scientific teaching (Handelsman *et al.*, 2007), we defined the broad and specific learning goals and then determined appropriate assessments to gauge learning gains for the specific goals (Figure 1A and Tables 1 and 2). Next, we designed an interactive primer design activity and instruction sheet to meet these goals (Supplemental Materials A and B). In addition, we used a practical research question to engage the students and present primer design in a realistic context. After completion of the unit, we used the assessments as a measure of student learning (Figures 1B, 2, and 3). We identified misconceptions based on their performance and, after our assessments were completed, revised the exercise and instruction sheet to provide more clarity. These revised versions are included as Supplemental Materials A and B.

Instructional design was guided by the 5E Instruction Model that organizes a teachable unit into components that engage students to

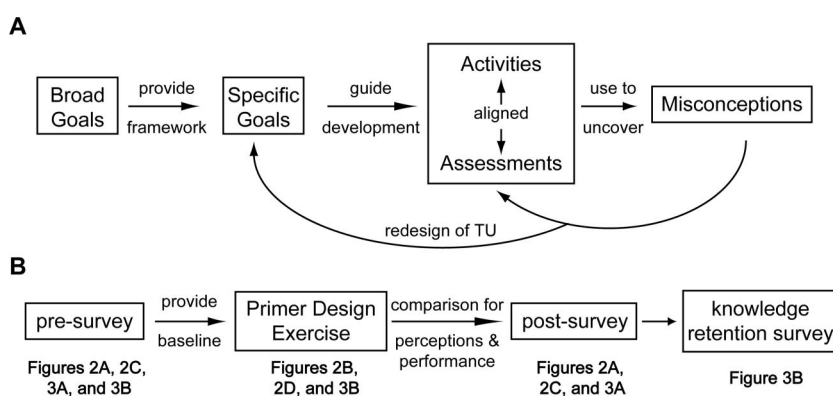


Figure 1. Alignment of the teachable unit. (A) Process of designing the teachable unit using backward design and scientific teaching principles. (B) Progression of assessments and activities. The figures that correspond with each activity and assessment is indicated in gray.

Table 2. Rubric for assessment of learning goals

Primer Design Exercise	Level 3	Level 2	Level 1
How well does the student understand the keys to a quality primer? (broad goals 1 and 2, specific goal 2)	The primers are designed to meet the criteria and the PCR reaction should work in theory.	The reaction might work, but there are potential problems with primer design or structure.	The reaction would not work due to poor primer design.
How well does the student understand the proper directionality/design of the reverse primer? (broad goals 1, 2, and 3, specific goal 1).	The 3' primer is the reverse complement of the sequence and should work in the reaction.	The primer is the reverse or the complement but not both and therefore the reaction will not work.	The primer is neither the complement nor the reverse and therefore the reaction will not work.
How well can the student use bioinformatics tools including BLAST searches to check primer quality and specificity? (broad goals 1, 2, and 4, specific goal 2).	The student has developed primers using bioinformatics that meet the criteria and the student clearly understands specificity of the primer.	The student can identify a problem with the primers using bioinformatics but cannot analyze the bioinformatics results correctly or completely.	The student does not use bioinformatics to check his/her primers or does not understand how the results indicate specificity of the primers to the target DNA.

explore, explain, and elaborate on a problem for evaluation (Biological Sciences Curriculum Study, 2003). The students were challenged to design primers to amplify a specific target sequence by PCR and were engaged in solving the problem. They explored DNA replication via PCR through online animations of PCR and by drawing several cycles of PCR by hand; see Phillips *et al.* (2008) in this issue for a more complete description of the teachable unit in which the Primer Design Exercise was implemented. Additionally, students utilized bioinformatics databases in the process of designing appropriate primers for PCR. Students had to explain their primer choices as well as the output of the database searches for primer specificity. Next, students elaborated on the design process by applying it to pre-selected primers used in the lab. Last, students were evaluated on their choice of primers and understanding of primer quality and specificity. Students had opportunities to approach the problem using diverse learning techniques including group and individual work, drawing to visualize primer annealing and directionality, and hands-on computer searches (Tanner and

Allen, 2004). The exercise highlights diverse organisms, including plant and bacterial examples of primer design, as well as multiple genomes from bacteria to humans, with regards to primer specificity. This allowed students to see the universality of the genetic code across living organisms and how one technique can be used in multiple contexts.

Class Activity

An essential component of PCR is designing quality primers, an exercise with which many undergraduates do not have much experience. To incorporate the theory of PCR into the lab, we developed a Primer Design Exercise that detailed the guidelines of a quality primer and gave the students the opportunity to design and test the quality of the primers they selected. Before starting the exercise, the students were given background on DNA replication during PCR and had already completed a graded exercise in which they drew

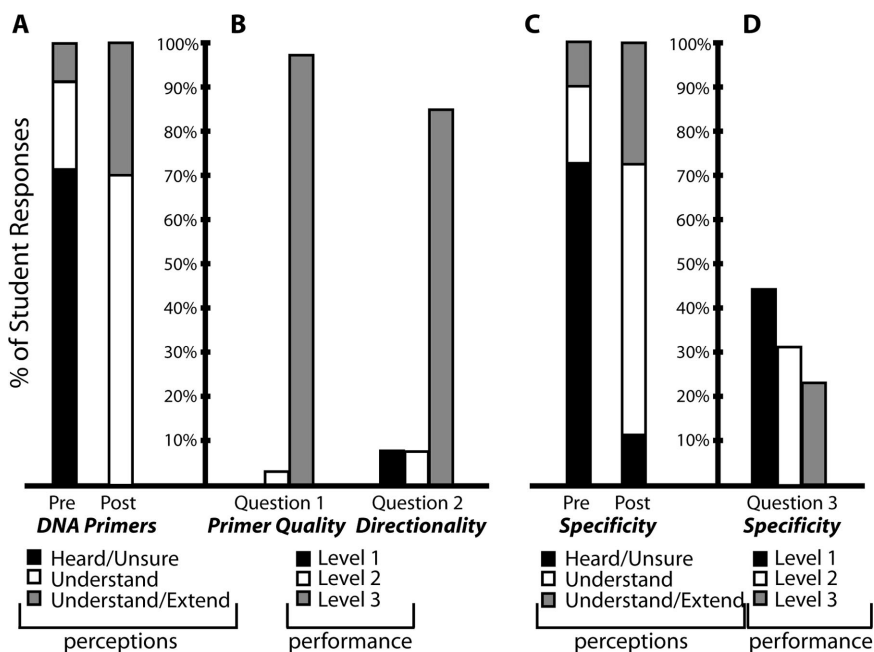


Figure 2. Students' perceptions and performance on the Primer Design Exercise. (A and C) Students' perceptions of understanding about (A) DNA primers and (C) BLAST search function. Columns represent student responses in pre- and postsurveys. ■, heard of the topic but unsure of the contextual meaning; □, understand the topic in the context of PCR; ▨, understand the topic and can extend understanding to other contexts. (B and D) Student performance. Question 1 (Primer Quality), How well does the student understand the keys to a quality primer? Question 2 (Directionality), How well does the student understand the proper directionality/design of the reverse primer? Question 3 (Specificity), How well can the students use bioinformatics tools, including BLAST searches, to check primer quality and specificity? Student understanding was ranked according to the rubric depicted in Table 2. All responses are presented as a percentage of total responses (n = 97).

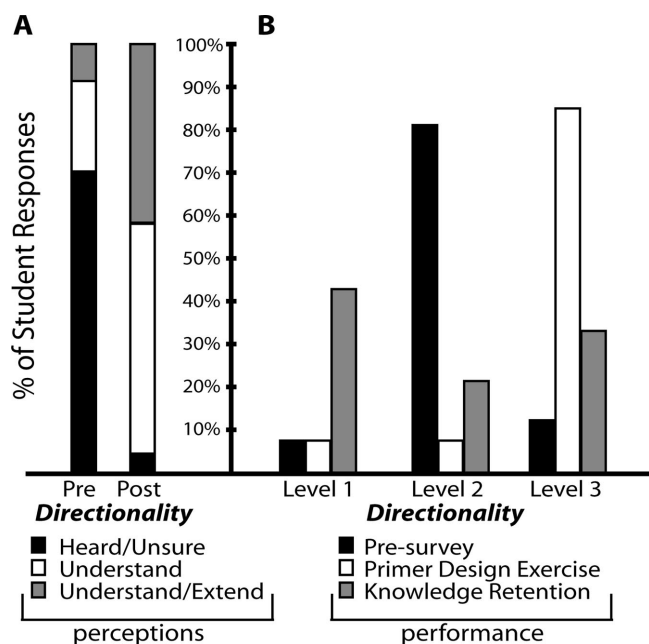


Figure 3. Student Learning Gains: directionality in primer design. (A) Students' perception of understanding about directionality of DNA. Columns represent student responses in pre- and postsurveys. ■, heard of the topic but unsure of the contextual meaning; □, understand the topic in the context of PCR; ▨, understand the topic and can extend understanding to other contexts. (B) Learning gains and knowledge retention. Students were asked on a presurvey to write the reverse complement of a DNA sequence and indicate directionality. As part of the Primer Design Exercise they had to create a primer that was the reverse complement of a DNA sequence and indicate directionality. Five months after completion of the unit, students were again asked to design a reverse primer for a DNA sequence and indicate proper directionality. Student understanding was ranked according to the rubric depicted in Table 2. All responses are presented as a percentage of total responses (n = 97).

the first three cycles of PCR. The exercise and answer key are available online at <http://scientificteaching.wisc.edu/materials/> and are described in more detail in Phillips *et al.* (2008). Drawing the cycles of PCR ensured that students were familiar with the replication process and was helpful in identifying misconceptions about DNA replication during PCR. In addition, students were given detailed instructions that described characteristics of quality primers such as appropriate annealing temperature, sequence specificity only to the DNA to be amplified, and no secondary structure. The instructions also outlined the steps to use the Basic Local Alignment Search Tool (BLAST) to test the specificity of the primer sequence in relation to the genome of interest (Supplemental Material A).

To model scientific research, the exercise was written in the context of a researcher trying to identify strains of the bacteria *Pseudomonas putida* that contain a homolog of a novel *aroA* gene that confers resistance to glyphosate, the active component in Roundup (Supplemental Material B; answer key is available online). The students were given the DNA sequence of the *P. putida aroA* gene and were asked to design forward and reverse primers, without the use of a computer program, to amplify a specific region by PCR. Students assessed the quality of their primers using a website (<http://www.sigma-genosys.com/calc/DNAcalc.asp>) that gave information about primer length, base composition, melting temperature, and secondary structure. Students made the decision to use their initial primers or redesign primers of a higher quality. Once good primers were designed, students used the BLAST func-

tion to determine the specificity of their primers (<http://www.ncbi.nlm.nih.gov/BLAST/>) to the *aroA* gene in *P. putida*. Finally, to connect the importance of primer design back to the wet lab, students were asked to use all available information to evaluate the primers that they were to use to amplify the constitutive Cauliflower Mosaic Virus (CaMV 35S) promoter to detect the presence of genetically modified organisms in food samples that the students brought to class.

Assessments

To assess student knowledge and retention, data were collected immediately before and after the teachable unit using electronic pre- and postsurveys and by a paper survey 5 months after completion of the unit (Figure 1B). Questions focused both on students' performance and perceptions of their understanding of DNA directionality, the steps of PCR, and bioinformatics tools such as BLAST. Student knowledge was assessed on the pre- and postsurveys by asking the students to write the reverse complement of a DNA sequence and indicate directionality. The retention assessment (5 months after the unit) asked students to design forward and reverse primers with proper directionality when given a DNA sequence. Students were given 10–15 minutes to complete the knowledge retention assessment without asking questions or using references.

An evaluative rubric for the instructors' use was designed to assess whether the students achieved specific learning goals, including understanding primer quality, directionality, and design and the use of bioinformatics tools to test primer specificity (Table 2). These questions covered multiple competence levels as outlined by Bloom's Taxonomy, including comprehension, analysis, and synthesis (Bloom, 1984). We used the broad and specific learning goals as a framework to design rubric questions. This process 1) ensured that the specific learning goals were being assessed by the questions on the exercise, 2) gave the instructors focal points to emphasize with the students during the unit, and 3) provided a standard to compare the pre- and postsurveys, the exercise, and retention questions (Huba and Freed, 2000; Handelsman *et al.*, 2007). After completion of the teachable unit, student responses were scored using a three-level scale, with a level three signifying the most complete answer. This evaluation was not included in the students' grades.

Class Structure

During the teachable unit, the students were given the exercise to work on in groups of four as time allowed during the lab. Students had full access to all materials and instruction sheets as well as lab computers and the Internet to complete the exercise. In addition, multiple instructors were available to answer questions. We found that the cursory coverage of PCR in lecture led to misconceptions and confusion with multiple molecular techniques and reagents. For example, students often confused the use of primers to amplify DNA in PCR and the use of restriction enzymes to cleave DNA in cloning. We encouraged the students to ask questions and engaged them in discussions to work through misconceptions and troubleshooting. Groups were formed randomly and often included at least one student experienced with PCR and/or primer design. Groups were actively engaged in the exercise and worked together to solve problems that promoted scientific communication and critical thinking (Ahern-Rindell, 1998).

RESULTS

Student understanding of quality, directionality, and specificity in primer design was assessed by students' performance and perceptions. Student performance on the Primer Design Exercise showed that a large majority of the students mastered two of the specific learning goals (Figure 2B). First, they were able to design quality primers with appropriate

annealing temperature, length, guanine-cytosine content, and no secondary structure or primer dimers (75%, level three). Second, they designed a reverse primer with proper directionality and complementarity (85%, level three). However, students struggled with the larger conceptual question of primer specificity to the target DNA of interest in relation to the whole genome (Figure 2D). Only 24% of students could analyze the data completely with regard to specificity. The implications of this misconception are discussed below.

Student understanding of DNA directionality was assessed multiple times over the course of the teachable unit (Figures 2B and 3). An assessment of student performance before the unit (presurvey) showed that most students obtained a level two understanding of primer directionality, indicating they were able to write the reverse or the complement of a DNA sequence but not both. However, on the Primer Design Exercise 85% of the students obtained a level three, meaning they designed primers that would allow the PCR reaction to work. Results of the retention survey, assessed 5 months after the unit, indicated that although some students could design appropriate primers (level 3), the majority of the students had trouble designing a reverse primer (Figure 3B).

Students' perceptions of their understanding of directionality were assessed in pre- and postsurveys (Figure 3A). Before the teachable unit 70% of the students had heard of directionality but were unsure of the contextual meaning. Only 7% felt they understood and could extend the concepts of directionality to primer design and other topics. After completion of the unit, 95% felt they understood the topic, with about half of those students reporting they could extend their knowledge to other contexts.

In addition, students' perceptions of their understanding of DNA primers and BLAST functions were also queried (Figure 2, A and C). As before, a majority of students reported on the presurvey they had heard of these topics but were unsure of the context. On completion of the unit, 100% of students reported an understanding of DNA primers and approximately 90% reported an understanding of BLAST searches. Many of these students also felt they could extend these topics to other contexts.

As part of the postsurvey, students were questioned on the usefulness of these materials in learning to design primers and perform BLAST searches. Sixty-three and 46% of students reported the primer design and BLAST search exercises, respectively, greatly contributed to their understanding of the technique. Thirty-three and 35%, respectively, found the exercises to somewhat help their understanding or clarify a misconception about the topic.

DISCUSSION

In teaching the theory of PCR and providing concrete examples of how to design biological reagents (primers), we hoped to provide students with a foundation to better troubleshoot, analyze, and interpret results from actual experiments. Although many students have exposure to or have utilized PCR in lab settings, we found that the majority do not understand how the process works or the importance of each reagent involved. Often, students are excited to learn a new molecular technique, such as PCR, but do not fully

appreciate the biological processes that make these techniques such powerful research tools. Previous instructors of the wet lab noted students had difficulty in describing aberrant PCR results. For example, students often relied on the ambiguous explanation of "contamination" to explain multiple bands or bands in the negative control on a DNA gel after PCR. We found that by incorporating the biological concepts of PCR with the fundamentals of primer design, students had a better understanding of the necessity of quality reagents and thus were more poised to address problems.

Student Performance

To assess the Primer Design Exercise, three assessment questions about primer quality, directionality, and specificity were based on the specific learning goals and evaluated using a rubric (Tables 1 and 2). As illustrated in Figures 2 and 3, students clearly grasped the concepts of primer quality and orientation but had more difficulty with the question regarding specificity and BLAST searches. Question 1 surveyed a comprehension level of student understanding of quality primers, as defined by Bloom's Taxonomy (Bloom, 1984). Students were able to read the criteria of quality primers and assess the quality of their own primers. Question 2 was an analysis-level question about primer directionality. Students initially struggled to visualize both strands of DNA and where each primer would bind. However, while completing the exercise, the students comprehended the concept of directionality by combining knowledge of DNA replication and base complementarity. Question 3 targeted synthesis of multiple sources of information. This question required higher-order thinking as students had to evaluate a single primer sequence in relation to multiple genomes, which may explain why more students struggled with the concept of specificity, ultimately exposing more misconceptions (see below).

Student perceptions of their understanding of DNA directionality, primer quality, and specificity corresponded well with measured student performance on the Primer Design Exercise (Figures 2 and 3). With regard to primer quality and DNA directionality, 95–100% of students reported that they understood these topics at the end of the exercise. This correlated with the majority of students achieving a level three of performance for questions corresponding to these topics. However, students were less confident in their ability to analyze the specificity of primers as it relates to the output of a BLAST search. This was evident in the misconceptions that were voiced and in their performance on specificity related questions.

Despite the incomplete understanding of specificity, we felt the specific student learning goals were met. By the end of the unit, students were clearly able to design primers using bioinformatics databases and had an intermediate understanding of specificity that iterations of the concept could clarify. Through hands-on problem solving, 37% (level three) of students retained a complete understanding of primer directionality, and 21% (level two) of students retained a partial understanding (Figure 2B). This indicated that, although the students had mastered primer design during the teachable unit, some of this knowledge was not retained. However, this initial exposure to primer design

laid the theoretical groundwork for further investigations using PCR primers or, by extension, other techniques involving DNA hybridization.

We believe that the testing environment for the retention question was significantly different from during the teachable unit because it was an individual task with no associated consequences such as the success or failure of their own experiment or receiving a grade. This may correlate with a reduced effort and seemingly less retention. It would also be interesting to further examine this idea of retention and whether it correlates to repeated exposure. For example, when discussing another molecular technique such as Northern blot hybridization, probe design should be highlighted to again discuss base complementarity and DNA directionality.

Identifying and Addressing Misconceptions

In addition to meeting the learning goals, we found several benefits to students designing their own primers. First, the students better understood the theory of how PCR is used to amplify a specific DNA sequence and why quality primers are so important, which helped to demystify the “magic” of PCR (Figure 2B). Second, students were better able to analyze their own results. A specific example of troubleshooting occurred when students in one section were mistakenly given a primer to use in the wet lab that had a single nucleotide error at the 3' end of the primer. When the lab technician realized the error, students were asked to explain how this error would affect the reaction and predict what their gel would look like. Through group discussion they were able to troubleshoot this problem and interpret the unexpected bands, which appeared on the gel. In the other four class sections we proposed the same problem of a single nucleotide change in the primer sequence and asked the students to predict the results of a PCR reaction with these primers. The students were able to draw the results on the board and explain why additional bands would appear on the gel. Additionally, the students were able to predict how an error at the 5' end of the primer might affect the reaction differently. Lastly, in designing their own primers, students exposed and often clarified their misconceptions about DNA replication, primer directionality, and primer specificity.

During the teachable unit, many misconceptions were identified by students and instructors. We had anticipated some of these from previous teaching experiences, but others were unexpected. The misconceptions were realized through various questions on the Primer Design Exercise. Supplemental Material C is an example of a student response to question 1 on the Primer Design Exercise that illustrates common misconceptions about DNA directionality, complementarity, primer annealing, and the DNA extension step of PCR. These misconceptions resulted in the design of primers that would not amplify the target sequence.

We observed that students had difficulty visualizing the second strand when given a single strand of a DNA sequence, which often led to designing both the sense and antisense primers as reverse complements of the sequence given. Many students did not fully grasp the concept until we asked them to write the base pair sequence, write the complementary strand, and identify 5' and 3' ends. It was through this formative assessment that the students made the connection between primer annealing and DNA exten-

sion. This process of active learning helped students recognize and clarify their misconceptions about DNA directionality and complementarity. Discussions with instructors or peers helped students to correct their misconceptions about primer directionality. This is evident in Figures 2 and 3, as the majority of students reached a level 3 understanding of primer directionality.

Students also struggled to understand the importance of specificity of the primer to the target sequence and how a BLAST search can indicate specificity. Students were able to compare their primers to a single organism, such as when analyzing BLAST results from the search for the *aroA* gene in *P. putida*. However, the students were less clear about the importance of specificity when asked to analyze search results for the CaMV 35S promoter primers from multiple organisms that may be present in their food products. They were also asked to think about contaminating DNA from their own hands. We observed that the students became confused when trying to differentiate between an exact alignment of what they were looking for, the source transgenic plants, and other relevant alignments that would indicate cross-reactivity. In addition, those who could identify other relevant alignments struggled to conceptualize the meaning of this alignment in relation to how their PCR results would appear on a gel and if additional bands could be explained by nonspecific priming. This more prevalent misconception is illustrated in Figure 2D, which shows that a significant portion of students remained at a level 1 of understanding. We were limited to one-on-one discussions about specificity and the meaning of BLAST results because time did not allow for a whole-class discussion; however, this may be more appropriate in the future. We revised the Primer Design Instruction Sheet in an attempt to clarify this topic, which is included as Supplemental Material A.

Transferability

Although designed to fit in the context of this lab, the exercise could easily be modified to be used in different settings such as a large classroom, independent research, or workshops as either an in-class or out-of-class activity. The Primer Design Instruction Sheet and Exercise are universal such that they could be applied to the amplification of any gene. We feel the design of this exercise could also be used as a template for designing other active-learning exercises that highlight experiment preparation and design, for example as with DNA or RNA probes used for Southern blots, Northern blots, in situ hybridization, or microarray.

Final Comments

We designed a Primer Design Exercise to complement the lab techniques that the students were concurrently learning. In doing so, students were able to correlate the biological principles involved in primer design and how primers affect PCR results. We used an active-learning approach to put the exercise in the context of amplifying a real gene for a defined experiment giving the students a sense of purpose. This lab exercise provided students with another realistic and diverse example of primer design, allowing them to build on existing knowledge, construct their own ideas, correct misconceptions, and get hands-on experience with this process.

We believed students were receptive to this hands-on approach to learning primer design because they were very engaged in the group activity and many voiced positive comments about this exercise. In this study we found that the concurrent lecture about PCR and primer design did not give students the opportunity to explore primer design and bioinformatics databases and construct their own ideas about primer directionality and specificity. One student stated, "I wish we would have done this exercise before we had to design primers from a protein sequence. I would have understood that lecture assignment better." In using active learning we were able to uncover many misconceptions about the topic and utilized group work to help clarify these misunderstandings. The rubric was designed to directly assess the specific learning goals and determine the effectiveness of the teachable unit. We found that the results of the rubric evaluation correlated well with student perceptions of how well they understood primer directionality, quality, and specificity. We hope the materials provided will benefit other instructors teaching these concepts.

Accessing Materials

The final Primer Design Instruction Sheet and Exercise are included as Supplemental Material and can also be accessed with the associated rubric and answer keys from the WPST Digital Library <http://scientificteaching.wisc.edu/materials>.

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